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(FILE 'HOME' ENTERED AT 09:44:07 ON 21 SEP 2004)  
SET COST OFF

FILE 'REGISTRY' ENTERED AT 09:44:26 ON 21 SEP 2004

E SMURF  
L1 20 S E3-E5 OR ?SMURF?/CNS  
E SMAD  
L2 403 S E3-E21

FILE 'HCAPLUS' ENTERED AT 09:46:07 ON 21 SEP 2004

L3 15 S L1  
L4 85 S ?SMURF?  
L5 90 S L3,L4  
L6 9 S L5 AND L2  
L7 48 S L5 AND ?SMAD?  
L8 50 S L6,L7  
L9 42 S L8 AND UBIQUITIN?  
L10 50 S L8,L9  
L11 7 S L10 AND SCREEN?  
E DRUG SCREENING/CT  
L12 24987 S E3-E5  
L13 6373 S E9,E10  
E E3+ALL  
L14 31124 S E9,E8  
E E12+ALL  
L15 9001 S E10  
L16 3897 S E21+NT  
L17 5 S L10 AND L12-L16  
L18 7 S L11,L17  
L19 8 S L10 AND ?MODULAT?  
L20 11 S L5 AND ?MODULAT?  
L21 15 S L18-L20  
L22 0 S L10 AND ?PPYX?  
L23 7 S L10 AND WW (L) DOMAIN  
L24 21 S L21,L23  
E WRANA J/AU  
L25 117 S E3-E9  
E THOMSEN G/AU  
L26 35 S E3-E6  
L27 9 S L25,L26 AND L5  
L28 25 S L24,L27  
L29 18 S L5 AND (PD<=19990611 OR PRD<=19990611 OR AD<=19990611)  
L30 2 S L28 AND L29  
L31 0 S L5 AND (PY<=1999 OR PRY<=1999 OR AY<=1999) NOT L29  
L32 9 S L27,L30  
L33 16 S L29 NOT L32  
SEL DN AN 2-9 11-16  
L34 2 S L33 NOT E1-E38  
L35 11 S L32,L34  
L36 3 S L3 AND L29  
L37 11 S L35,L36

=> fil hcaplus

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FILE COVERS 1907 - 21 Sep 2004 VOL 141 ISS 13  
FILE LAST UPDATED: 20 Sep 2004 (20040920/ED)

This file contains CAS Registry Numbers for easy and accurate substance identification.

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L37 ANSWER 1 OF 11 HCAPLUS COPYRIGHT 2004 ACS on STN  
AN 2003:941411 HCAPLUS  
DN 140:3090  
ED Entered STN: 03 Dec 2003  
TI Regulation of Cell Polarity and Protrusion Formation by Targeting RhoA for Degradation  
AU Wang, Hong-Rui; Zhang, Yue; Ozdamar, Barish; Ogunjimi, Abiodun A.; Alexandrova, Evguenia; Thomsen, Gerald H.; Wrana, Jeffrey L.  
CS Samuel Lunenfeld Research Institute, Program in Molecular Biology and Cancer, Mount Sinai Hospital, Toronto, M56 1X5, Can.  
SO Science (Washington, DC, United States) (2003), 302(5651), 1775-1779  
CODEN: SCIEAS; ISSN: 0036-8075  
PB American Association for the Advancement of Science  
DT Journal  
LA English  
CC 13-2 (Mammalian Biochemistry)  
AB The Rho family of small guanosine triphosphatases regulates actin cytoskeleton dynamics that underlie cellular functions such as cell shape changes, migration, and polarity. We found that **Smurf1**, a HECT domain E3 ubiquitin ligase, regulated cell polarity and protrusive activity and was required to maintain the transformed morphol. and motility of a tumor cell. Atypical protein kinase C zeta (PKC $\zeta$ ), an effector of the Cdc42/Rac1-PAR6 polarity complex, recruited **Smurf1** to cellular protrusions, where it controlled the local level of RhoA. **Smurf1** thus links the polarity complex to degradation of RhoA in lamellipodia and filopodia to prevent RhoA signaling during dynamic membrane movements.  
ST **Smurf1** C kinase actin RhoA degrdn cell polarity morphol  
IT Rho protein (G protein)  
RL: BSU (Biological study, unclassified); BIOL (Biological study) (RhoA; regulation of cell polarity and protrusion formation by targeting RhoA for degradation)  
IT Cell morphology  
Cytoskeleton  
(regulation of actin cytoskeleton dynamics and cell polarity and protrusion formation by targeting RhoA for degradation)  
IT Actins  
RL: BSU (Biological study, unclassified); BIOL (Biological study) (regulation of actin cytoskeleton dynamics and cell polarity and protrusion formation by targeting RhoA for degradation)  
IT Protein degradation  
(regulation of cell polarity and protrusion formation by targeting RhoA for degradation)  
IT 74812-49-0, E3 Ubiquitin ligase  
RL: BSU (Biological study, unclassified); BIOL (Biological study)

(Smurf1; protein kinase C $\zeta$  recruits Smurf1 to cellular protrusions, where it controls local level of RhoA)  
 IT 472998-88-2, Protein kinase C $\zeta$   
 RL: BSU (Biological study, unclassified); BIOL (Biological study)  
 (protein kinase C $\zeta$  recruits Smurf1 to cellular protrusions, where it controls local level of RhoA)

RE.CNT 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD  
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L37 ANSWER 2 OF 11 HCAPLUS COPYRIGHT 2004 ACS on STN

AN 2003:813733 HCAPLUS

DN 140:126177

ED Entered STN: 16 Oct 2003

TI The RING-H2 protein RNF11 is overexpressed in breast cancer and is a target of Smurf2 E3 ligase

AU Subramaniam, V.; Li, H.; Wong, M.; Kitching, R.; Attisano, L.; Wrana, J.; Zubovits, J.; Burger, A. M.; Seth, A.

CS CIHR Group in Matrix Dynamics, Sunnybrook and Women's College Health Sciences Centre, 1Laboratory of Molecular Pathology and Molecular and Cellular Biology Research, University of Toronto, Toronto, ON, Can.

SO British Journal of Cancer (2003), 89(8), 1538-1544

CODEN: BJCAAI; ISSN: 0007-0920

PB Nature Publishing Group

DT Journal

LA English

CC 14-1 (Mammalian Pathological Biochemistry)

AB The breast cancer-associated T2A10 clone was originally isolated from a cDNA library enriched for tumor messenger ribonucleic acids. Our survey of 125

microarrayed primary tumor tissues using affinity purified polyclonal antibodies has revealed that corresponding protein is overexpressed in invasive breast cancer and is weakly expressed in kidney and prostate tumors. Now known as RNF11, the gene encodes a RING-H2 domain and a PY motif, both of which mediate protein-protein interactions. In particular, the PPPPY sequence of RNF11 PY motif is identical to that of Smad7, which has been shown to bind to WW domains of **Smurf2**, an E3 ubiquitin ligase that mediates the ubiquitination and degradation of the TGF $\beta$  receptor complex. Using various mutants of RNF11 in GST pulldown and immunopptn. assays, we found that RNF11 interacts with **Smurf2** through the PY motif, leading to ubiquitination of both proteins. **Smurf2** plays an active role in the repression of TGF $\beta$  signaling, and our data indicate that overexpression of RNF11, through its interaction with **Smurf2**, can restore TGF $\beta$  responsiveness in transfected cells.

ST **Smurf2** RNF11 protein breast cancer

IT Protein motifs

(PY motif, of RNF11; RING-H2 protein RNF11 is overexpressed in breast cancer and is target of **Smurf2** E3 ligase)

IT Bladder, neoplasm

Head, neoplasm

Lung, neoplasm

(RING-H2 protein RNF11 expression in)

IT Human

Mammary gland, neoplasm

Molecular association

Signal transduction, biological

(RING-H2 protein RNF11 is overexpressed in breast cancer and is target of **Smurf2** E3 ligase)

IT Proteins

RL: BSU (Biological study, unclassified); BIOL (Biological study)

(RNF11; RING-H2 protein RNF11 is overexpressed in breast cancer and is target of **Smurf2** E3 ligase)

IT Protein motifs

(WW domains, of **Smurf2**; RING-H2 protein RNF11 is overexpressed in breast cancer and is target of **Smurf2** E3 ligase)

IT Pancreas, neoplasm

(adenocarcinoma; RING-H2 protein RNF11 expression in)

IT Prostate gland, neoplasm

(carcinoma; RING-H2 protein RNF11 expression in)

IT Intestine, neoplasm

(colon; RING-H2 protein RNF11 expression in)

IT Neck, anatomical

(neoplasm; RING-H2 protein RNF11 expression in)

IT Kidney, neoplasm

(renal cell carcinoma; RING-H2 protein RNF11 expression in)

IT Enzymes, biological studies

RL: BSU (Biological study, unclassified); BIOL (Biological study)

(ubiquitin-conjugating, UBCH5A; RING-H2 protein RNF11 is overexpressed in breast cancer and is target of **Smurf2** E3 ligase)

IT Transforming growth factors

RL: BSU (Biological study, unclassified); BIOL (Biological study)

( $\beta$ -; RING-H2 protein RNF11 is overexpressed in breast cancer and is target of **Smurf2** E3 ligase)

IT Transforming growth factor receptors

RL: BSU (Biological study, unclassified); BIOL (Biological study)

( $\beta$ -transforming growth factor; RING-H2 protein RNF11 is overexpressed in breast cancer and is target of **Smurf2** E3 ligase)

IT 74812-49-0, E3 Ubiquitin ligase

RL: BSU (Biological study, unclassified); BIOL (Biological study)

(RING-H2 protein RNF11 is overexpressed in breast cancer and is target

of Smurf2 E3 ligase)

IT 60267-61-0, Ubiquitin

RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(ubiquitination; RING-H2 protein RNF11 is overexpressed in breast  
cancer and is target of Smurf2 E3 ligase)

RE.CNT 30 THERE ARE 30 CITED REFERENCES AVAILABLE FOR THIS RECORD  
RE

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L37 ANSWER 3 OF 11 HCAPLUS COPYRIGHT 2004 ACS on STN

AN 2003:493891 HCAPLUS

DN 140:315556

ED Entered STN: 30 Jun 2003

TI Distinct endocytic pathways regulate TGF- $\beta$  receptor signalling and  
turnover [Erratum to document cited in CA139:191931]

AU Di'guglielmo, Gianni M.; Le Roy, Christine; Goodfellow, Anne F.;  
**Wrana, Jeffrey L.**

CS Samuel Lunenfeld Research Institute, Programme in Molecular Biology and  
Cancer, Mount Sinai Hospital, Toronto, ON, Can.

SO Nature Cell Biology (2003), 5(7), 680

CODEN: NCBIFN; ISSN: 1465-7392

PB Nature Publishing Group

DT Journal

LA English

CC 2-10 (Mammalian Hormones)

AB On page 414, second column, line 9, the text should read "Autoradiog.  
(Fig. 1g) and quantitation (Fig. 1h)..." rather than "Autoradiog. (Fig.  
1g) and quantitation (Fig. 1g)..."

ST erratum TGFbeta receptor endocytosis endosome vesicle lipid caveolae  
signaling; endocytosis TGF beta receptor signaling turnover erratum

IT Clathrin

RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(-dependent endocytosis; distinct endocytic pathways regulate

- TGF- $\beta$  receptor signaling and turnover as studied in mammalian cells (Erratum))
- IT Lipids, biological studies  
 RL: BSU (Biological study, unclassified); BIOL (Biological study) (-rafts on cell membrane; distinct endocytic pathways regulate TGF- $\beta$  receptor signaling and turnover as studied in mammalian cells (Erratum))
- IT Antigens  
 RL: BSU (Biological study, unclassified); BIOL (Biological study) (EEA-1 (early endosome antigen-1); distinct endocytic pathways regulate TGF- $\beta$  receptor signaling and turnover as studied in mammalian cells (Erratum))
- IT Proteins  
 RL: BSU (Biological study, unclassified); BIOL (Biological study) (SARA (Smad anchor for receptor activation); distinct endocytic pathways regulate TGF- $\beta$  receptor signaling and turnover as studied in mammalian cells (Erratum))
- IT Transcription factors  
 RL: BSU (Biological study, unclassified); BIOL (Biological study) (Smad-2; distinct endocytic pathways regulate TGF- $\beta$  receptor signaling and turnover as studied in mammalian cells (Erratum))
- IT Transcription factors  
 RL: BSU (Biological study, unclassified); BIOL (Biological study) (Smad-7; distinct endocytic pathways regulate TGF- $\beta$  receptor signaling and turnover as studied in mammalian cells (Erratum))
- IT Proteins  
 RL: BSU (Biological study, unclassified); BIOL (Biological study) (Smurf2; distinct endocytic pathways regulate TGF- $\beta$  receptor signaling and turnover as studied in mammalian cells (Erratum))
- IT Transforming growth factor receptors  
 RL: BSU (Biological study, unclassified); BIOL (Biological study) (TGF- $\beta$  receptor, type I; distinct endocytic pathways regulate TGF- $\beta$  receptor signaling and turnover as studied in mammalian cells (Erratum))
- IT Transforming growth factor receptors  
 RL: BSU (Biological study, unclassified); BIOL (Biological study) (TGF- $\beta$  receptor, type II; distinct endocytic pathways regulate TGF- $\beta$  receptor signaling and turnover as studied in mammalian cells (Erratum))
- IT Organelle  
 (caveolae; distinct endocytic pathways regulate TGF- $\beta$  receptor signaling and turnover as studied in mammalian cells (Erratum))
- IT Proteins  
 RL: BSU (Biological study, unclassified); BIOL (Biological study) (caveolins; distinct endocytic pathways regulate TGF- $\beta$  receptor signaling and turnover as studied in mammalian cells (Erratum))
- IT Organelle  
 (coated pit; distinct endocytic pathways regulate TGF- $\beta$  receptor signaling and turnover as studied in mammalian cells (Erratum))
- IT Endocytosis  
 Endosome  
 Human  
 Protein degradation  
 Signal transduction, biological  
 (distinct endocytic pathways regulate TGF- $\beta$  receptor signaling and turnover as studied in mammalian cells (Erratum))
- IT Organelle  
 (endocytic vesicle, early; distinct endocytic pathways regulate TGF- $\beta$  receptor signaling and turnover as studied in mammalian cells (Erratum))
- IT Biological transport  
 (internalization; distinct endocytic pathways regulate TGF- $\beta$

receptor signaling and turnover as studied in mammalian cells (Erratum))

IT Cell membrane  
(lipid rafts on-; distinct endocytic pathways regulate TGF- $\beta$  receptor signaling and turnover as studied in mammalian cells (Erratum))

IT Phosphorylation, biological  
(protein; distinct endocytic pathways regulate TGF- $\beta$  receptor signaling and turnover as studied in mammalian cells (Erratum))

IT Transforming growth factors  
RL: BSU (Biological study, unclassified); BIOL (Biological study) ( $\beta$ -; distinct endocytic pathways regulate TGF- $\beta$  receptor signaling and turnover as studied in mammalian cells (Erratum))

IT 74812-49-0  
RL: BSU (Biological study, unclassified); BIOL (Biological study) (**Smurf2**; distinct endocytic pathways regulate TGF- $\beta$  receptor signaling and turnover as studied in mammalian cells (Erratum))

IT 60267-61-0, Ubiquitin  
RL: BSU (Biological study, unclassified); BIOL (Biological study) (distinct endocytic pathways regulate TGF- $\beta$  receptor signaling and turnover as studied in mammalian cells (Erratum))

L37 ANSWER 4 OF 11 HCAPLUS COPYRIGHT 2004 ACS on STN  
AN 2003:332595 HCAPLUS  
DN 139:191931  
ED Entered STN: 01 May 2003  
TI Distinct endocytic pathways regulate TGF- $\beta$  receptor signalling and turnover  
AU Di Guglielmo, Gianni M.; Le Roy, Christine; Goodfellow, Anne F.; **Wrana, Jeffrey L.**  
CS Samuel Lunenfeld Research Institute, Programme in Molecular Biology and Cancer, Mount Sinai Hospital, Toronto, Can.  
SO Nature Cell Biology (2003), 5(5), 410-421  
CODEN: NCBIFN; ISSN: 1465-7392  
PB Nature Publishing Group  
DT Journal  
LA English  
CC 2-10 (Mammalian Hormones)  
AB Endocytosis of cell surface receptors is an important regulatory event in signal transduction. The transforming growth factor  $\beta$  (TGF- $\beta$ ) superfamily signals to the Smad pathway through heteromeric Ser-Thr kinase receptors that are rapidly internalized and then downregulated in a ubiquitin-dependent manner. TGF- $\beta$  receptors internalize into both caveolin- and EEA1-pos. vesicles and reside in both lipid raft and non-raft membrane domains. Clathrin-dependent internalization into the EEA1-pos. endosome, where the Smad2 anchor SARA is enriched, promotes TGF- $\beta$  signaling. In contrast, the lipid raft-caveolar internalization pathway contains the Smad7-**Smurf2** bound receptor and is required for rapid receptor turnover. Thus, segregation of TGF- $\beta$  receptors into distinct endocytic compartments regulates Smad activation and receptor turnover.

ST TGF beta receptor endocytosis endosome vesicle lipid caveolae signaling  
IT Clathrin  
RL: BSU (Biological study, unclassified); BIOL (Biological study) (-dependent endocytosis; distinct endocytic pathways regulate TGF- $\beta$  receptor signalling and turnover as studied in mammalian cells)

IT Lipids, biological studies  
RL: BSU (Biological study, unclassified); BIOL (Biological study) (-rafts on cell membrane; distinct endocytic pathways regulate TGF- $\beta$  receptor signalling and turnover as studied in mammalian cells)

- IT Antigens
  - RL: BSU (Biological study, unclassified); BIOL (Biological study) (EEA-1 (early endosome antigen-1); distinct endocytic pathways regulate TGF- $\beta$  receptor signalling and turnover as studied in mammalian cells)
- IT Proteins
  - RL: BSU (Biological study, unclassified); BIOL (Biological study) (SARA (Smad anchor for receptor activation); distinct endocytic pathways regulate TGF- $\beta$  receptor signalling and turnover as studied in mammalian cells)
- IT Transcription factors
  - RL: BSU (Biological study, unclassified); BIOL (Biological study) (Smad-2; distinct endocytic pathways regulate TGF- $\beta$  receptor signalling and turnover as studied in mammalian cells)
- IT Transcription factors
  - RL: BSU (Biological study, unclassified); BIOL (Biological study) (Smad-7; distinct endocytic pathways regulate TGF- $\beta$  receptor signalling and turnover as studied in mammalian cells)
- IT Proteins
  - RL: BSU (Biological study, unclassified); BIOL (Biological study) (Smurf2; distinct endocytic pathways regulate TGF- $\beta$  receptor signalling and turnover as studied in mammalian cells)
- IT Transforming growth factor receptors
  - RL: BSU (Biological study, unclassified); BIOL (Biological study) (TGF- $\beta$  receptor, type I; distinct endocytic pathways regulate TGF- $\beta$  receptor signalling and turnover as studied in mammalian cells)
- IT Transforming growth factor receptors
  - RL: BSU (Biological study, unclassified); BIOL (Biological study) (TGF- $\beta$  receptor, type II; distinct endocytic pathways regulate TGF- $\beta$  receptor signalling and turnover as studied in mammalian cells)
- IT Organelle
  - (caveolae; distinct endocytic pathways regulate TGF- $\beta$  receptor signalling and turnover as studied in mammalian cells)
- IT Proteins
  - RL: BSU (Biological study, unclassified); BIOL (Biological study) (caveolins; distinct endocytic pathways regulate TGF- $\beta$  receptor signalling and turnover as studied in mammalian cells)
- IT Organelle
  - (coated pit; distinct endocytic pathways regulate TGF- $\beta$  receptor signalling and turnover as studied in mammalian cells)
- IT Endocytosis
  - Endosome
  - Human
  - Protein degradation
  - Signal transduction, biological
    - (distinct endocytic pathways regulate TGF- $\beta$  receptor signalling and turnover as studied in mammalian cells)
- IT Organelle
  - (endocytic vesicle, early; distinct endocytic pathways regulate TGF- $\beta$  receptor signalling and turnover as studied in mammalian cells)
- IT Biological transport
  - (internalization; distinct endocytic pathways regulate TGF- $\beta$  receptor signalling and turnover as studied in mammalian cells)
- IT Cell membrane
  - (lipid rafts on-; distinct endocytic pathways regulate TGF- $\beta$  receptor signalling and turnover as studied in mammalian cells)
- IT Phosphorylation, biological
  - (protein; distinct endocytic pathways regulate TGF- $\beta$  receptor signalling and turnover as studied in mammalian cells)
- IT Transforming growth factors

RL: BSU (Biological study, unclassified); BIOL (Biological study)  
( $\beta$ -; distinct endocytic pathways regulate TGF- $\beta$  receptor  
signalling and turnover as studied in mammalian cells)

IT 74812-49-0, Ubiquitin ligase

RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(Smurf2; distinct endocytic pathways regulate TGF- $\beta$   
receptor signalling and turnover as studied in mammalian cells)

IT 60267-61-0, Ubiquitin

RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(distinct endocytic pathways regulate TGF- $\beta$  receptor signalling  
and turnover as studied in mammalian cells)

RE.CNT 50 THERE ARE 50 CITED REFERENCES AVAILABLE FOR THIS RECORD  
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 AN 2001:453687 HCAPLUS  
 DN 135:162954  
 ED Entered STN: 24 Jun 2001  
 TI TGF- $\beta$  induces assembly of a Smad2- **Smurf2** ubiquitin ligase complex that targets SnoN for degradation  
 AU Bonni, Shirin; Wang, Hong-Rui; Causing, Carrie G.; Kavsak, Peter; Stroschein, Shannon L.; Luo, Kunxin; **Wrana, Jeffrey L.**  
 CS Program in Molecular Biology and Cancer, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, M5G 1X5, Can.  
 SO Nature Cell Biology (2001), 3(6), 587-595  
 CODEN: NCBIFN; ISSN: 1465-7392  
 PB Nature Publishing Group  
 DT Journal  
 LA English  
 CC 2-10 (Mammalian Hormones)  
 AB The receptor-regulated Smad proteins are essential intracellular mediators of signal transduction by the transforming growth factor- $\beta$  (TGF- $\beta$ ) superfamily of growth factors and are also important as regulators of gene transcription. Here we describe a new role for TGF- $\beta$ -regulated Smad2 and Smad3 as components of a ubiquitin ligase complex. We show that in the presence of TGF- $\beta$  signaling, Smad2 interacts through its proline-rich PPXY motif with the tryptophan-rich WW domains of **Smurf2**, a recently identified E3 ubiquitin ligases. TGF- $\beta$  also induces the association of **Smurf2** with the transcriptional-co-repressor SnoN and we show that Smad2 can function to mediate this interaction. This allows **Smurf2** HECT domain to target SnoN for ubiquitin-mediated degradation by the proteasome. Thus, stimulation by TGF- $\beta$  can induce the assembly of a Smad2-**Smurf2** ubiquitin ligase complex that functions to target substrates for degradation  
 ST TGF Smad2 **Smurf2** ubiquitin ligase SnoN  
 IT Transcription factors  
 RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)  
 (Smad-2; TGF- $\beta$  induces assembly of Smad2- **Smurf2** ubiquitin ligase complex that targets SnoN for degradation)  
 IT Transforming proteins  
 RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)  
 (SnoN; TGF- $\beta$  induces assembly of Smad2- **Smurf2** ubiquitin ligase complex that targets SnoN for degradation)  
 IT Signal transduction, biological  
 (TGF- $\beta$  induces assembly of Smad2- **Smurf2** ubiquitin ligase complex that targets SnoN for degradation)  
 IT Transforming growth factors  
 RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)  
 ( $\beta$ -; TGF- $\beta$  induces assembly of Smad2- **Smurf2** ubiquitin ligase complex that targets SnoN for degradation)  
 IT 74812-49-0, Synthetase, ubiquitin-protein 140879-24-9, Proteasome  
 RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)  
 (TGF- $\beta$  induces assembly of Smad2- **Smurf2** ubiquitin ligase complex that targets SnoN for degradation)  
 RE.CNT 39 THERE ARE 39 CITED REFERENCES AVAILABLE FOR THIS RECORD  
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- L37 ANSWER 6 OF 11 HCAPLUS COPYRIGHT 2004 ACS on STN  
AN 2001:59228 HCAPLUS  
DN 134:233168  
ED Entered STN: 25 Jan 2001  
TI Smad7 binds to **Smurf2** to form an E3 ubiquitin ligase that targets the TGF $\beta$  receptor for degradation  
AU Kavsak, Peter; Rasmussen, Richele K.; Causing, Carrie G.; Bonni, Shirin; Zhu, Haitao; **Thomsen, Gerald H.**; **Wrana, Jeffrey L.**  
CS Program in Molecular Biology and Cancer Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, M5G 1X5, Can.  
SO Molecular Cell (2000), 6(6), 1365-1375  
CODEN: MOCEFL; ISSN: 1097-2765  
PB Cell Press  
DT Journal  
LA English  
CC 6-1 (General Biochemistry)  
Section cross-reference(s): 7  
AB Ubiquitin-mediated proteolysis regulates the activity of diverse receptor systems. Here, we identify **Smurf2**, a C2-WW-HECT domain ubiquitin ligase and show that **Smurf2** assoc. constitutively with Smad7. **Smurf2** is nuclear, but binding to Smad7 induces export and recruitment to the activated TGF $\beta$  receptor, where it causes degradation of receptors and Smad7 via proteasomal and lysosomal pathways. IFN $\gamma$ , which stimulates expression of Smad7, induces Smad7-**Smurf2** complex formation and increases TGF $\beta$  receptor turnover, which is stabilized by blocking Smad7 or **Smurf2** expression. Furthermore, Smad7 mutants that interfere with recruitment of **Smurf2** to the receptors are compromised in their inhibitory

activity. These studies thus define Smad7 as an adaptor in an E3 ubiquitin-ligase complex that targets the TGF $\beta$  receptor for degradation

ST Smad7 **Smurf2** E3 ubiquitin ligase TGFbeta receptor degrdn;

IT transforming growth factor beta receptor degrdn ubiquitin ligase Smad7

IT Transcription factors

RL: BAC (Biological activity or effector, except adverse); BOC (Biological occurrence); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); OCCU (Occurrence); PROC (Process)

(Smad-7, adaptor protein; Smad7 binds to **Smurf2** to form an E3 ubiquitin ligase that targets the TGF $\beta$  receptor for degradation)

IT Molecular association

(Smad7 binds to **Smurf2** to form an E3 ubiquitin ligase that targets the TGF $\beta$  receptor for degradation)

IT Cell nucleus

(**Smurf2** export; Smad7 binds to **Smurf2** to form an E3 ubiquitin ligase that targets the TGF $\beta$  receptor for degradation)

IT Cytoplasm

(cytosol, **Smurf2** export from cell nucleus to cytosol; Smad7 binds to **Smurf2** to form an E3 ubiquitin ligase that targets the TGF $\beta$  receptor for degradation)

IT Biological transport

(export, of **Smurf2** from cell nucleus; Smad7 binds to **Smurf2** to form an E3 ubiquitin ligase that targets the TGF $\beta$  receptor for degradation)

IT Transforming growth factor receptors

RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)

( $\beta$ -transforming growth factor; Smad7 binds to **Smurf2** to form an E3 ubiquitin ligase that targets the TGF $\beta$  receptor for degradation)

IT Interferons

RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)

( $\gamma$ ; Smad7 binds to **Smurf2** to form an E3 ubiquitin ligase that targets the TGF $\beta$  receptor for degradation)

IT 60267-61-0, Ubiquitin

RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)

(Smad7 binds to **Smurf2** to form an E3 ubiquitin ligase that targets the TGF $\beta$  receptor for degradation)

IT 74812-49-0, E3 Ubiquitin ligase

RL: BAC (Biological activity or effector, except adverse); BOC (Biological occurrence); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); OCCU (Occurrence); PROC (Process)

(**Smurf2**; Smad7 binds to **Smurf2** to form an E3 ubiquitin ligase that targets the TGF $\beta$  receptor for degradation)

RE.CNT 35 THERE ARE 35 CITED REFERENCES AVAILABLE FOR THIS RECORD

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L37 ANSWER 7 OF 11 HCAPLUS COPYRIGHT 2004 ACS on STN

AN 2000:900772 HCAPLUS

DN 134:53133

ED Entered STN: 22 Dec 2000

TI sequences human **ubiquitin**-protein synthetases as antagonists of  
BMP and TGF $\beta$  signaling pathways and expression during development and  
interactions with **Smad** proteins

IN **Thomsen, Gerald H.; Wrana, Jeffrey**

PA Research Foundation of State University of New York, USA; HSC Research and  
Development Limited Partnership

SO PCT Int. Appl., 106 pp.

CODEN: PIXXD2

DT Patent

LA English

IC ICM C12N

CC 7-2 (Enzymes)

Section cross-reference(s): 12, 13

FAN.CNT 1

|      | PATENT NO.      | KIND   | DATE     | APPLICATION NO. | DATE         |
|------|-----------------|--|----------|-----------------|--------------|
| PI   | WO 2000077168   | A2   | 20001221 | WO 2000-US16250 | 20000612 <-- |
|      | WO 2000077168   | A3   | 20010503 |                 |              |
|      | W:              | AE, AG, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, MZ, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM |          |                 |              |
|      | RW:             | GH, GM, KE, LS, MW, MZ, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG   |          |                 |              |
|      | AU 2000056107   | A5   | 20010102 | AU 2000-56107   | 20000612 <-- |
|      | EP 1192174      | A2   | 20020403 | EP 2000-941398  | 20000612 <-- |
|      | R:              | AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, SI, LT, LV, FI, RO   |          |                 |              |
| PRAI | US 1999-138969P | P  | 19990611 | <--             |              |
|      | WO 2000-US16250 | W  | 20000612 |                 |              |

CLASS

| PATENT NO. | CLASS | PATENT FAMILY CLASSIFICATION CODES |
|------------|-------|------------------------------------|
|------------|-------|------------------------------------|

|               |     |      |
|---------------|-----|------|
| WO 2000077168 | ICM | C12N |
|---------------|-----|------|

AB This invention provides unique members of the Hect family of

ubiquitin ligases that specifically target BMP and TGF $\beta$ /activin pathway-specific Smads. The novel ligases have been named Smurf1 and Smurf2. A transgenic expression system is described for these two proteins. They directly interact with Smads1 and 5 and Smad7, resp., and regulate the ubiquitination, turnover and activity of Smads and other proteins of these pathways. Smurf1 interferes with biol. responses to BMP, but not activin signaling. In amphibian embryos Smurf1 inhibits endogenous BMP signals, resulting in altered pattern formation and cell fate specification in the mesoderm and ectoderm. The present invention provides a unique regulatory link between the ubiquitination pathway and the control of cell fate determination by the TGF $\beta$  superfamily during embryonic development. Thus, Smurf1 is a neg. regulator of Smad1 signal transduction, by targeting Smad1, Smurf1 blocks BMP signaling. Screening assays which survey Smurf WW domain interaction with Smad protein PPXY domain are also relayed. In mammalian cells, Smurf2 suppresses TGF $\beta$  signaling, and in Xenopus, blocks formation of dorsal mesoderm and causes anterior truncation of the embryos. Smurf2 forms a stable complex with Smad7, which induces degradation and downregulation of TGF $\beta$ /activin signaling. The human Smurf1 gene was mapped to 7q21.1-q31.1.

- ST human Smurf1 Smurf2 cDNA sequence BMP TGF $\beta$  signaling development; ubiquitin protein synthetase sequence human Smurf1 Smurf2
- IT Probes (nucleic acid)  
 RL: ARG (Analytical reagent use); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)  
 (DNA probe identifying Smurf genes; sequences human ubiquitin-protein synthetases as antagonists of BMP and TGF $\beta$  signaling pathways and expression during development and interactions with Smad proteins)
- IT Nucleic acid hybridization  
 (DNA-DNA, DNA probe identifying Smurf genes; sequences human ubiquitin-protein synthetases as antagonists of BMP and TGF $\beta$  signaling pathways and expression during development and interactions with Smad proteins)
- IT Proteins, specific or class  
 RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)  
 (Smad7; Smurf1 and Smurf3 ligase interactions with; sequences human ubiquitin-protein synthetases as antagonists of BMP and TGF $\beta$  signaling pathways and expression during development and interactions with Smad proteins)
- IT Proteins, specific or class  
 RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)  
 (Smads5; Smurf1 and Smurf3 ligase interactions with; sequences human ubiquitin-protein synthetases as antagonists of BMP and TGF $\beta$  signaling pathways and expression during development and interactions with Smad proteins)
- IT Proteins, specific or class  
 RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)  
 (Smads; Smurf1 and Smurf3 ligase interactions with; sequences human ubiquitin-protein synthetases as antagonists of BMP and TGF $\beta$  signaling pathways and expression during development and interactions with Smad proteins)
- IT Bone morphogenetic proteins  
 RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL

(Biological study); PROC (Process)

(**Smurf1** inhibiting BMP alternating mesoderm and ectoderm; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

IT Mutation

(**Smurf1** or **Smurf2** mutation occurring at C710A or C716A; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

IT Protein degradation

(**Smurf2** induces degradation of TGFbeta receptors and **Smad7**; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

IT Gene, animal

RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)

(**Smurf2**; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

IT Protein motifs

(assay for **screening Smurf WW domain** interaction with **Smad** protein PPXY domain; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways)

IT Embryo, animal

(blastula, **Smurf1** mRNA localization to blastula; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

IT Proteins, specific or class

RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)

(complexes, **Smurf2** forming stable complex with **Smad7**; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

IT Development, nonmammalian postembryonic

(developmental gene expression of **Smurf1**; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

IT Embryo, animal

(ectoderm, **Smurf1** inhibiting BMP alternating mesoderm and ectoderm; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

IT Gene

(expression, developmental gene expression of **Smurf1**; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

IT Antibodies

RL: ARG (Analytical reagent use); BSU (Biological study, unclassified); ANST (Analytical study); BIOL (Biological study); USES (Uses)

(identifying **Smurf** proteins; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

IT Embryo, animal

(mesoderm, **Smurf1** inhibiting BMP alternating mesoderm and

ectoderm; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

- IT Genetic mapping  
Genetic vectors  
Protein sequences  
Transformation, genetic  
Xenopus laevis  
cDNA sequences  
(sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)
- IT Gene, animal  
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)  
(**smurf1**; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)
- IT Transforming growth factors  
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)  
( $\beta$ -; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)
- IT 74812-49-0, Synthetase, **ubiquitin**-protein  
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)  
(**Smurf1** and **Smurf2** as; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)
- IT 314013-12-2 314013-13-3  
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)  
(amino acid sequence; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)
- IT 312903-81-4 314013-11-1  
RL: BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study)  
(nucleotide sequence; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)
- IT 60267-61-0, **Ubiquitin**  
RL: BSU (Biological study, unclassified); BIOL (Biological study)  
(sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)
- IT 314014-74-9, 5: PN: WO0077168 PAGE: 47 unclaimed DNA 314014-75-0, 6: PN: WO0077168 PAGE: 48 unclaimed DNA  
RL: PRP (Properties)  
(unclaimed nucleotide sequence; sequences human **ubiquitin**-protein synthetases as antagonists of BMP and TGF<b signaling pathways and expression during development and interactions with **Smad** proteins)

ED Entered STN: 21 Apr 2000  
 TI Ubiquitin protein ligase-target-binding protein fusion and method for  
 targeted proteolysis  
 IN Zhou, Pengbo; Howley, Peter  
 PA President and Fellows of Harvard College, USA  
 SO PCT Int. Appl., 185 pp.  
 CODEN: PIXXD2

DT Patent

LA English

IC ICM C12N015-00

ICS C12N015-62; C12N015-12; C12N015-37; C12N015-52; C12N005-10;  
 C07K014-00

CC 9-2 (Biochemical Methods)

FAN.CNT 1

|      | PATENT NO.      | KIND   | DATE         | APPLICATION NO. | DATE         |
|------|-----------------|--|--------------|-----------------|--------------|
| PI   | WO 2000022110   | A2   | 20000420     | WO 1999-US23705 | 19991008 <-- |
|      | WO 2000022110   | A3   | 20001116     |                 |              |
|      | W:              | AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, UZ, VN, YU, ZA, ZW, AM, AZ, BY, KG, KZ, MD, RU, TJ, TM |              |                 |              |
|      | RW:             | GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW, AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE, BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG   |              |                 |              |
| PRAI | US 1998-103787P | P  | 19981009 <-- |                 |              |

CLASS

| PATENT NO.    | CLASS | PATENT FAMILY CLASSIFICATION CODES                                     |
|---------------|-------|--|
| WO 2000022110 | ICM   | C12N015-00   |
|               | ICS   | C12N015-62; C12N015-12; C12N015-37; C12N015-52; C12N005-10; C07K014-00 |
| WO 2000022110 | ECLA  | C07K014/025; C07K014/47; C07K014/47A24; C12N009/00L; C12N015/62 <--    |

AB The present invention relates to methods and reagents for targeting proteolysis of a polypeptide by cis or trans association with a ubiquitin protein ligase. Methods and reagents for inhibiting the ubiquitination and proteolysis of cellular proteins which are recognized by a ubiquitin protein ligase are also disclosed. Thus, a gene encoding  $\beta$ TrCP fused to papillomavirus E7 oncoprotein N-terminus and a gene encoding the Rb protein were expressed in human osteosarcoma Saos-2 cells lacking the Rb protein. Although the Rb protein is normally stable in this cell, the protein was rapidly degraded in the transformant.

ST E3 ubiquitin ligase fusion targeted proteolysis

IT Peptidomimetics

(E3 ubiquitin ligase antagonizing; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT Peptides, biological studies

RL: THU (Therapeutic use); BIOL (Biological study); USES (Uses)  
 (E3 ubiquitin ligase antagonizing; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT Proteins, specific or class

RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)  
 (E6-AP, fusions with target protein-binding domains; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT Transcription factors

- RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)  
(E7, target protein-binding domain of, fusions with E3 ubiquitin ligase; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
- IT Transcription factors  
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)  
(IkB; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
- IT Transcription factors  
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)  
(Rb, p107; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
- IT Transcription factors  
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)  
(Rb; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
- IT Proteins, specific or class  
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)  
(cullin, fusions with target protein-binding domains; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
- IT Chimeric gene  
RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)  
(for E3 ubiquitin ligase-targeting domain fusion; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
- IT Proteins, specific or class  
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)  
(gene CDC4, fusions with target protein-binding domains; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
- IT Transcription factors  
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)  
(gene E2, of human papillomavirus 16; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
- IT Proteins, specific or class  
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)  
(gene EDD, fusions with target protein-binding domains; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
- IT Proteins, specific or class  
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)  
(gene FWD1, fusions with target protein-binding domains; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
- IT Proteins, specific or class

- RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)  
(gene HOS, fusions with target protein-binding domains; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
- IT Proteins, specific or class  
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)  
(gene RSP5, fusions with target protein-binding domains; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
- IT Proteins, specific or class  
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)  
(gene Smurf1, fusions with target protein-binding domains; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
- IT Proteins, specific or class  
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)  
(gene TOM1, fusions with target protein-binding domains; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
- IT Proteins, specific or class  
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)  
(gene UBR1, fusions with target protein-binding domains; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
- IT Proteins, specific or class  
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)  
(gene cln2; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
- IT Proteins, specific or class  
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)  
(gene grr1, fusions with target protein-binding domains; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
- IT Proteins, specific or class  
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)  
(gene met30, fusions with target protein-binding domains; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
- IT Proteins, specific or class  
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)  
(gene nedd-4, fusions with target protein-binding domains; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
- IT Proteins, specific or class  
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL

- (Biological study); PREP (Preparation)  
(gene pop1, fusions with target protein-binding domains; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
- IT Proteins, specific or class  
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)  
(gene pop2, fusions with target protein-binding domains; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
- IT Proteins, specific or class  
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)  
(gene sic1; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
- IT Antigens  
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)  
(large T, target protein-binding domain of, fusions with E3 ubiquitin ligase; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
- IT Protein degradation  
(targeted; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
- IT Proteins, general, biological studies  
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)  
(ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
- IT Catenins  
RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)  
( $\beta$ -; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
- IT Proteins, specific or class  
RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)  
( $\beta$ TrCP, fusions with target protein-binding domains; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
- IT 143550-97-4, Protein (Saccharomyces cerevisiae clone pBM1720 gene GGR1 reduced) 169539-01-9 207624-75-7 211629-03-7 235430-49-6 264185-29-7  
RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)  
(amino acid sequence; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
- IT 111116-07-5, DNA (Saccharomyces cerevisiae gene CDC4) 140735-75-7, GenBank M59247 202318-98-7, GenBank AF038867 206092-47-9, GenBank Y14153 225436-29-3, GenBank AF081887 264185-28-6  
RL: BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); USES (Uses)  
(nucleotide sequence; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)
- IT 140879-24-9, Proteasome  
RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)  
(targeting proteins for degradation by; ubiquitin protein ligase-target-binding protein fusion and method for targeted

proteolysis)

IT 74812-49-ODP, E3 Ubiquitin ligase, fusions with target protein-binding domains  
 RL: BAC (Biological activity or effector, except adverse); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BIOL (Biological study); PREP (Preparation)  
 (ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT 255361-87-6, 1: PN: WO0001720 PAGE: 41 unclaimed DNA 255361-88-7, 5: PN: WO0001720 PAGE: 41 unclaimed DNA 255701-25-8, 9: PN: WO0001720 PAGE: 42 unclaimed DNA 264594-21-0, 7: PN: WO0022110 PAGE: 141 unclaimed DNA 264594-22-1, 8: PN: WO0022110 PAGE: 142 unclaimed DNA 264594-23-2 264594-24-3 264594-25-4 264594-26-5 264594-27-6 264594-28-7 264594-29-8 264594-30-1 264594-31-2 264594-32-3 264594-33-4 264594-34-5 264594-35-6 264863-83-4, 9: PN: WO0022110 PAGE: 142 unclaimed DNA  
 RL: PRP (Properties)  
 (unclaimed nucleotide sequence; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT 124585-34-8, 1-72-RNA formation factor (human immunodeficiency virus 1 clone pCV-1/pPL-12 gene tat reduced) 255385-06-9 264594-20-9  
 RL: PRP (Properties)  
 (unclaimed protein sequence; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

IT 255039-62-4  
 RL: PRP (Properties)  
 (unclaimed sequence; ubiquitin protein ligase-target-binding protein fusion and method for targeted proteolysis)

L37 ANSWER 9 OF 11 HCAPLUS COPYRIGHT 2004 ACS on STN  
 AN 2000:234998 HCAPLUS  
 DN 132:329985  
 ED Entered STN: 12 Apr 2000  
 TI The Smad pathway  
 AU Wrana, Jeffrey L.; Attisano, Liliana  
 CS Program in Molecular Biology and Cancer, Samuel Lunenfeld Research Institute, Mount Sinai Hospital, Toronto, ON, M5G 1X5, Can.  
 SO Cytokine & Growth Factor Reviews (2000), 11(1/2), 5-13  
 CODEN: CGFRFB; ISSN: 1359-6101  
 PB Elsevier Science Ltd.  
 DT Journal; General Review  
 LA English  
 CC 2-0 (Mammalian Hormones)  
 AB A review with 54 refs. Transforming growth factor- $\beta$  superfamily member signals are conveyed through cell-surface serine/threonine kinase receptors to the intracellular mediators known as Smads. Activation of Smads causes their translocation from the cytoplasm to the nucleus where they function to control gene expression. In this review the authors will focus on proteins that modulate Smad activity, including SARA, for Smad Anchor for Receptor Activation, which functions during the initiation of signaling and on components of the ubiquitin-proteasome pathway, such as Smurf1, which can neg. regulate Smad signaling. In addition, the authors will summarize recent findings on the role of Smads as transcriptional co-modulators.

ST review Smad protein signal transduction TGF beta  
 IT Signal transduction, biological  
 (Smad signaling pathway in relation to proteins that modulate Smad activity and components of the ubiquitin-proteasome pathway)

IT Transcription factors  
 RL: BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); PROC (Process)  
 (Smad; Smad signaling pathway in relation to proteins that modulate Smad activity and components of the ubiquitin-proteasome pathway)

IT Transforming growth factors

RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)

( $\beta$ -; Smad signaling pathway in relation to proteins that modulate Smad activity and components of the ubiquitin-proteasome pathway)

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AN 1999:544811 HCAPLUS  
 DN 131:284125  
 ED Entered STN: 30 Aug 1999  
 TI A SMAD ubiquitin ligase targets the BMP pathway and affects embryonic pattern formation  
 AU Zhu, Haitao; Kavsak, Peter; Abdollah, Shirin; Wrana, Jeffrey L.; Thomsen, Gerald H.  
 CS Department of Biochemistry and Cell Biology and Institute for Cell and Developmental Biology, State University of New York, Stony Brook, NY, 11794-5215, USA  
 SO Nature (London) (1999), 400(6745), 687-693  
 CODEN: NATUAS; ISSN: 0028-0836  
 PB Macmillan Magazines  
 DT Journal  
 LA English  
 CC 12-3 (Nonmammalian Biochemistry)  
 Section cross-reference(s): 3, 7  
 AB TGF- $\beta$ -like factors signal across cell membranes through complexes of transmembrane receptors known as type I and type II serine/threonine-kinase receptors, which in turn activate the SMAD signaling pathway. On the inside of the cell membrane, a receptor-regulated class of SMADs are phosphorylated by the type-I-receptor kinase. In this way, receptors for different factors are able to pass on specific signals along the pathway: for example, receptors for bone morphogenetic protein (BMP) target SMADs 1, 5, and 8, whereas receptors for activin and TGF- $\beta$  target SMADs 2 and 3. Phosphorylation of receptor-regulated SMADs induces their association with Smad4, the common-partner SMAD, and stimulates accumulation of this complex in the nucleus, where it regulates transcriptional responses. Here we describe *Smurf1*, a new member of the Hect family of E3 ubiquitin ligases. *Smurf1* selectively interacts with receptor-regulated SMADs specific for the BMP pathway to trigger their ubiquitination and degradation, and hence their inactivation. In the amphibian *Xenopus laevis*, *Smurf1* mRNA is localized to the animal pole of the egg; in *Xenopus* embryos, ectopic *Smurf1* inhibits the transmission of BMP signals and thereby affects pattern formation. *Smurf1* also enhances cellular responsiveness to the Smad2 (activin/TGF- $\beta$ ) pathway. Thus, targeted ubiquitination of SMADs may serve to control both embryonic development and a wide variety of cellular responses to TGF- $\beta$  signals.  
 ST frog embryo signaling ubiquitin ligase; sequence E3 ubiquitin ligase  
 Xenopus  
 IT Cell nucleus  
 Development, nonmammalian postembryonic  
 Egg  
 Eye  
 Kidney  
 Molecular cloning  
 Protein sequences  
 Signal transduction, biological  
 Transcriptional regulation  
*Xenopus laevis*  
 cDNA sequences  
 (SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)  
 IT Bone morphogenetic proteins  
 RL: BAC (Biological activity or effector, except adverse); BSU (Biological study, unclassified); BIOL (Biological study)  
 (SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)  
 IT Bone morphogenetic protein receptors  
 RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)  
 (SMAD ubiquitin ligase sequence and targeting of BMP pathway and role

in embryonic pattern formation in frogs)

- IT Transcription factors  
 RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)  
 (Smad-1; SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)
- IT Transcription factors  
 RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)  
 (Smad-2; SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)
- IT Transcription factors  
 RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)  
 (Smad-5; SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)
- IT Gene, animal  
 RL: BOC (Biological occurrence); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence); PROC (Process)  
 (Smurf1; SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)
- IT Embryo, animal  
 (branchial arch; SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)
- IT Nervous system  
 (central; SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)
- IT Embryo, animal  
 (ectoderm; SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)
- IT Embryo, animal  
 (embryogenesis; SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)
- IT Gene  
 (expression; SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)
- IT Embryo, animal  
 (mesoderm; SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)
- IT Phosphorylation, biological  
 (protein; SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)
- IT Embryo, animal  
 (somite; SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)
- IT 74812-49-0, E3 Ubiquitin ligase  
 RL: BAC (Biological activity or effector, except adverse); BOC (Biological occurrence); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence); PROC (Process)  
 (SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)
- IT **246223-04-1**  
 RL: BAC (Biological activity or effector, except adverse); BOC (Biological occurrence); BPR (Biological process); BSU (Biological study, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence); PROC (Process)  
 (amino acid sequence; SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)

IT 237728-87-9, GenBank AF169310

RL: PRP (Properties)

(nucleotide sequence; SMAD ubiquitin ligase sequence and targeting of BMP pathway and role in embryonic pattern formation in frogs)

RE.CNT 31 THERE ARE 31 CITED REFERENCES AVAILABLE FOR THIS RECORD

RE

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- (29) Ward, C; Cell 1995, V83, P121 HCAPLUS
- (30) Whitman, M; Genes Dev 1998, V12, P2445 HCAPLUS
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L37 ANSWER 11 OF 11 HCAPLUS COPYRIGHT 2004 ACS on STN

AN 1997:335178 HCAPLUS

DN 126:303135

ED Entered STN: 29 May 1997

TI Eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses

IN Beach, David; Caligiuri, Maureen; Nefsky, Bradley

PA Cold Spring Harbor Laboratory, USA; Beach, David; Caligiuri, Maureen; Nefsky, Bradley

SO PCT Int. Appl., 109 pp.

CODEN: PIXXD2

DT Patent

LA English

IC ICM C12N009-00

ICS C12Q001-25

CC 7-2 (Enzymes)

Section cross-reference(s): 3, 10, 13

FAN.CNT 1

|    | PATENT NO.   | KIND | DATE     | APPLICATION NO. | DATE         |
|----|--|------|----------|-----------------|--------------|
| PI | WO 9712962   | A1   | 19970410 | WO 1996-US15930 | 19961004 <-- |
|    | W: CA, JP, MX, US  |      |          |                 |              |
|    | RW: AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE |      |          |                 |              |
|    | US 6001619   | A    | 19991214 | US 1995-539205  | 19951004 <-- |

|   |    |          |                 |              |
|---|----|----------|-----------------|--------------|
| CA 2231645  | AA | 19970410 | CA 1996-2231645 | 19961004 <-- |
| EP 857205   | A1 | 19980812 | EP 1996-933244  | 19961004 <-- |
| R: AT, BE, CH, DE, DK, ES, FR, GB, GR, IT, LI, LU, NL, SE, MC, PT, IE, FI |    |          |                 |              |
| US 6503742  | B1 | 20030107 | US 1999-392163  | 19990908 <-- |
| US 2003199036   | A1 | 20031023 | US 2002-313955  | 20021205 <-- |
| PRAI US 1995-539205   | A  | 19951004 | <--             |              |
| WO 1996-US15930   | W  | 19961004 | <--             |              |
| US 1999-392163  | A1 | 19990908 |                 |              |

## CLASS

| PATENT NO.    | CLASS  | PATENT FAMILY CLASSIFICATION CODES  |     |
|---------------|--|-------------------------------------|-----|
| WO 9712962    | ICM  | C12N009-00                          |     |
|               | ICS  | C12Q001-25                          |     |
| US 6001619    | ECLA   | C12N009/00L; G01N033/50D2           | <-- |
| US 6503742    | ECLA   | C12N009/00L; C12Q001/25; G01N033/68 | <-- |
| US 2003199036 | ECLA   | C12N009/00L; C12Q001/25; G01N033/68 | <-- |
| AB            | <p>The present invention relates to the discovery in eukaryotic cells of ubiquitin ligases. These proteins are referred to herein collectively as "pub" proteins for Protein Ubiquitin ligase, and individually as h-pub1, h-pub2, h-pub3 and s-pub1 for the human pub1, pub2 an pub3 and Schizosaccharomyces pombe pub1 clones, resp. Pub1 proteins apparently play a role in the ubiquitination of the mitotic activating tyrosine phosphatase cdc25, and thus they may regulate the progression of proliferation in eukaryotic cells by activating the cyclin dependent kinase complexes. In S. pombe, disruption of s-pub1 elevates the level of cdc25 protein in vivo increasing the activity of the tyrosine kinases. Wee1 and mik1, required to arrest the cell-cycle. Loss of wee1 function in an S. pombe cell carrying a disruption in the s-pub1 gene results in a lethal premature entry into mitosis; such lethal phenotype can be rescued by the loss of cdc25 function. A ubiquitin thioester adduct of s-pub1 can be isolated from S. pombe and disruption of s-pub1 dramatically reduces ubiquitination of cdc25.</p> |                                     |     |
| ST            | <p>ubiquitin ligase gene pub Schizosaccharomyces human; cDNA sequence<br/>ubiquitin ligase Schizosaccharomyces human; tyrosine phosphatase cdc25<br/>ubiquitin ligase pub; kinase tyrosine wee1 mik1 ubiquitin ligase; mitosis<br/>ubiquitin ligase pub Schizosaccharomyces human</p>  |                                     |     |
| IT            | <p>Genetic mapping<br/>(Schizosaccharomyces pombe gene pub1 mapping on chromosome 1 right arm; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)</p>   |                                     |     |
| IT            | <p>Cell proliferation<br/>Eukaryote (Eukaryotae)<br/>Mitosis<br/>Protein sequences<br/>Schizosaccharomyces pombe<br/>cDNA sequences<br/>(eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)</p>   |                                     |     |
| IT            | <p>Genetic vectors<br/>(expression; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)</p>  |                                     |     |
| IT            | <p>Proteins, specific or class<br/>RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)<br/>(gene wee1, activation by cdc25; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)</p>  |                                     |     |
| IT            | <p>Chromosome<br/>(microbial, Schizosaccharomyces pombe gene pub1 mapping on chromosome 1</p>  |                                     |     |

right arm; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)

IT Cell cycle

(progression; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)

IT Gene, animal

Gene, microbial

RL: BOC (Biological occurrence); BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); OCCU (Occurrence); PROC (Process); USES (Uses)

(pub1; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)

IT Gene, animal

RL: BOC (Biological occurrence); BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); OCCU (Occurrence); PROC (Process); USES (Uses)

(pub2; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)

IT Gene, animal

RL: BOC (Biological occurrence); BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); OCCU (Occurrence); PROC (Process); USES (Uses)

(pub3; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)

IT Animal

(transgenic, expression host; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)

IT Enzymes, biological studies

RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)

(ubiquitin-activating, ubiquitin conjugation system use; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)

IT Enzymes, biological studies

RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)

(ubiquitin-conjugating, ubiquitin conjugation system use; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)

IT p53 (protein)

RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)

(ubiquitination; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)

IT Fusion proteins (chimeric proteins)

RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)

(with ubiquitin ligase; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)

IT 144114-10-3, Protein kinase mik1

- RL: BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)  
(activation by cdc25; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)
- IT 189284-44-4P 189284-45-5P 189284-46-6P 189284-47-7P  
RL: BAC (Biological activity or effector, except adverse); BOC (Biological occurrence); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence); PREP (Preparation); USES (Uses)  
(amino acid sequence; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)
- IT 74812-49-0P, Ubiquitin-protein ligase  
RL: BAC (Biological activity or effector, except adverse); BOC (Biological occurrence); BPN (Biosynthetic preparation); BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence); PREP (Preparation); USES (Uses)  
(eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)
- IT 60267-61-0D, Ubiquitin, gene pub1 ubiquitin ligase thioester adducts  
74812-49-0D, Ubiquitin-protein ligase, ubiquitin thioester adducts  
RL: BOC (Biological occurrence); BSU (Biological study, unclassified); MFM (Metabolic formation); BIOL (Biological study); FORM (Formation, nonpreparative); OCCU (Occurrence)  
(eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)
- IT 189284-40-0 189284-41-1 189284-42-2 189284-43-3  
RL: BOC (Biological occurrence); BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); PRP (Properties); BIOL (Biological study); OCCU (Occurrence); PROC (Process); USES (Uses)  
(nucleotide sequence; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)
- IT 9000-83-3, ATPase 60267-61-0, Ubiquitin  
RL: BPR (Biological process); BSU (Biological study, unclassified); BUU (Biological use, unclassified); BIOL (Biological study); PROC (Process); USES (Uses)  
(ubiquitin conjugation system use; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)
- IT 140208-22-6, Gene cdc25 phosphatase  
RL: BAC (Biological activity or effector, except adverse); BPR (Biological process); BSU (Biological study, unclassified); BIOL (Biological study); PROC (Process)  
(ubiquitination; eukaryote gene pub1, pub2, and pub3 ubiquitin ligases, enzyme function in cell cycle progression, human and Schizosaccharomyces pombe cDNA sequences, and uses)

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FILE RELOADED: 19 October 2003.

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L44 ANSWER 1 OF 1 BIOSIS COPYRIGHT (c) 2004 The Thomson Corporation. on STN  
 AN 1999:431362 BIOSIS  
 DN PREV199900431362  
 TI A **SMAD** ubiquitin ligase targets the BMP pathway and affects  
 embryonic pattern formation.  
 AU Zhu, Haitao; Kavsak, Peter; Abdollah, Shirin; Wrana, Jeffrey L.;  
 Thomsen, Gerald H. [Reprint author]  
 CS Department of Biochemistry and Cell Biology and Institute for Cell and  
 Developmental Biology, State University of New York, Stony Brook, NY,  
 11794-5215, USA  
 SO Nature (London), (Aug. 12, 1999) Vol. 400, No. 6745, pp. 687-693. print.  
 CODEN: NATUAS. ISSN: 0028-0836.  
 DT Article  
 LA English  
 ED Entered STN: 18 Oct 1999  
 Last Updated on STN: 18 Oct 1999  
 AB The TGF-beta superfamily of proteins regulates many different biological  
 processes, including cell growth, differentiation and embryonic pattern  
 formation. TGF-beta-like factors signal across cell membranes through  
 complexes of transmembrane receptors known as type I and type II  
 serine/threonine-kinase receptors, which in turn activate the **SMAD**  
 signalling pathway. On the inside of the cell membrane, a  
 receptor-regulated class of **SMADs** are phosphorylated by the  
 type-I-receptor kinase. In this way, receptors for different factors are  
 able to pass on specific signals along the pathway: for example, receptors  
 for bone morphogenetic protein (BMP) target **SMADs** 1, 5 and 8,  
 whereas receptors for activin and TGF-beta target **SMADs** 2 and 3.  
 Phosphorylation of receptor-regulated **SMADs** induces their  
 association with **Smad4**, the 'common-partner' **SMAD**, and  
 stimulates accumulation of this complex in the nucleus, where it regulates  
 transcriptional responses. Here we describe **Smurf1**, a new  
 member of the Hect family of E3 ubiquitin ligases. **Smurf1**  
 selectively interacts with receptor-regulated **SMADs** specific for  
 the BMP pathway in order to trigger their ubiquitination and degradation,  
 and hence their inactivation. In the amphibian *Xenopus laevis*,  
**Smurf1** messenger RNA is localized to the animal pole of the egg;  
 in *Xenopus* embryos, ectopic **Smurf1** inhibits the transmission of  
 BMP signals and thereby affects pattern formation. **Smurf1** also  
 enhances cellular responsiveness to the **Smad2** (activin/TGF-beta)  
 pathway. Thus, targeted ubiquitination of **SMADs** may serve to  
 control both embryonic development and a wide variety of cellular  
 responses to TGF-beta signals.  
 CC Cytology - Animal 02506  
 Biochemistry methods - General 10050  
 Biochemistry studies - General 10060  
 Biophysics - General 10502  
 Enzymes - General and comparative studies: coenzymes 10802  
 Development and Embryology - General and descriptive 25502  
 General biology - Miscellaneous 00532  
 IT Major Concepts  
 Cell Biology; Development; Enzymology (Biochemistry and Molecular  
 Biophysics)  
 IT Chemicals & Biochemicals  
 bone morphogenetic protein [BMP]; **SMAD** ubiquitin ligase: BMP  
 pathway targeting  
 IT Miscellaneous Descriptors  
 embryonic pattern formation  
 ORGN Classifier

Cercopithecidae 86205  
 Super Taxa  
 Primates; Mammalia; Vertebrata; Chordata; Animalia  
 Organism Name  
 COS-1 cell line: African green monkey cells  
 Taxa Notes  
 Animals, Chordates, Mammals, Nonhuman Mammals, Nonhuman Vertebrates,  
 Nonhuman Primates, Primates, Vertebrates  
 ORGN Classifier  
 Mammalia 85700  
 Super Taxa  
 Vertebrata; Chordata; Animalia  
 Organism Name  
 293T cell line: mammalian cells  
 Taxa Notes  
 Animals, Chordates, Mammals, Nonhuman Vertebrates, Nonhuman Mammals,  
 Vertebrates  
 ORGN Classifier  
 Salientia 85306  
 Super Taxa  
 Amphibia; Vertebrata; Chordata; Animalia  
 Organism Name  
 Xenopus  
 Taxa Notes  
 Amphibians, Animals, Chordates, Nonhuman Vertebrates, Vertebrates

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L46 ANSWER 1 OF 7 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN

AN 2004-625495 [60] WPIX

DNC C2004-225008

TI Decreasing infection of cell by virus, HIV, influenza A or Ebola,  
 comprises interfering with activity or expression of host proteins or  
 activity of host nucleic acids such as Rab9, AXL receptor tyrosine kinase,  
 and Beta-chimerin .

DC B04 D16  
 IN HODGE, T W; MOREY, N J; RUBIN, D; SANCHEZ, A; SHAW, M W  
 PA (USSH) US DEPT HEALTH & HUMAN SERVICES  
 CYC 107  
 PI WO 2004070002 A2 20040819 (200460)\* EN 396 C12N000-00  
 RW: AT BE BG BW CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE  
 LS LU MC MW MZ NL OA PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW  
 W: AE AG AL AM AT AU AZ BA BB BG BR BW BY BZ CA CH CN CO CR CU CZ DE  
 DK DM DZ EC EE EG ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG  
 KP KR KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NI NO NZ OM  
 PG PH PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG US  
 UZ VC VN YU ZA ZM ZW  
 ADT WO 2004070002 A2 WO 2003-US37143 20031118  
 PRAI US 2003-482604P 20030625; US 2002-427464P 20021118  
 IC ICM C12N000-00  
 AB WO2004070002 A UPAB: 20040920  
 NOVELTY - Decreasing infection of a host cell by a virus comprises  
 interfering with an activity or expression of one or more host proteins or  
 interfering with an activity of one or more host nucleic acids where the  
 host protein or nucleic acid comprises Rab9, AXL receptor tyrosine kinase,  
 Beta-chimerin and mammalian selenium binding protein.  
 DETAILED DESCRIPTION - INDEPENDENT CLAIMS are included for the  
 following:  
 (1) methods of decreasing HIV, Ebola, or influenza A infection of a  
 host cell;  
 (2) a method of treating an HIV, Ebola, or influenza A viral  
 infection in a host subject;  
 (3) a method of determining resistance or susceptibility to viral  
 infection in a subject;  
 (4) a method of identifying a compound that decreases binding of a  
 viral protein to a host protein and decreases viral infection;  
 (5) a method of decreasing infection of a host cell by a pathogen;  
 (6) a cell comprising a functional deletion of one or more target  
 sequences associated with any of the 35 nucleotide sequences fully defined  
 in the specification, where the cell has a decreased susceptibility to HIV  
 infection;  
 (7) a cell comprising a functional deletion of one or more target  
 sequences associated with any of the 27 nucleotide sequences fully defined  
 in the specification, where the cell has a decreased susceptibility to  
 influenza infection;  
 (8) a cell comprising a functional deletion of one or more target  
 sequences associated with any of the 168 nucleotide sequences fully  
 defined in the specification, where the cell has a decreased  
 susceptibility to Ebola infection;  
 (9) a cell comprising a functional deletion of a Rab9 gene, where the  
 cell has a decreased susceptibility to infection by a pathogen that uses  
 lipid rafts; and  
 (10) a non-human transgenic mammal comprising any of the functional  
 deletions cited above.  
 ACTIVITY - Virucide; Anti-HIV; Antibacterial.  
 MECHANISM OF ACTION - RNAi; RNA interference; Axl tryosine kinase  
 receptor inhibitor; Rab9 inhibitor; beta chimerin inhibitor;  
 retinoblastoma binding protein 1 inhibitor; protein cell control  
 modulator; mammalian selenium binding protein inhibitor; KOX inhibitor.  
 Rab9, AXL (AXL receptor tyrosine kinase), CHN (Beta-chimerin), KOX,  
 RBB (retinoblastoma binding protein 1), KIAA1259, F3 and mammalian  
 selenium binding protein siRNA sequences were generated, pooled,  
 hybridized to its appropriate complement sequence and used to transfect  
 JC53 (HeLa cells modified to accept HIV), Vero (monkey kidney cells), MDCK  
 (dog kidney cells, or HEK (human kidney cells). GFP siRNA sequences were  
 used as negative controls.  
 Cells (20000 to 250000) were incubated in serum free media for 24  
 hours. Cocktails were made by mixing the siRNAs (50-100 pmoles) with

lipofectamine 2000 (4-16 micro l) and RNase inhibitor (1-4 micro l) in a solution of Optimem (serum free medium) in a total volume of 200-2000 micro l. Aliquots (50-500 micro l) of the cocktail were added to the cells which were incubated at 37 deg. C for 48 hours. The cells were then infected with HIV, Ebola, or influenza and the incubation continued for 3-7 days. Following transfection, several assays were conducted to confirm transfection efficiency and to determine the resistance of the cells to infection by various agents.

Quantitation of p24 levels of HIV infected J5C3 cells was determined. Rab9 siRNAs and mammalian selenium binding protein siRNAs each decreased HIV infection by 50% on day 4 post infection (day 7 post addition of siRNA). In addition, HIV infection decreased by 80-90% in the presence of beta-chimerin siRNAs, KOX siRNAs, or retinoblastoma binding protein 1 siRNA. However, HIV infection did not decrease in the presence of siRNAs that recognize KIAA1259, F3 or AXL siRNAs.

Infection of Ebola in HEK293 cells transfected with Rab9 or AXL siRNA was determined by measuring gp1 antigen using fluorescent antibody to gp1 envelope protein. Infection was decreased by 90-95% in presence of Rab9 siRNA, as compared to infection in absence of Rab9. Infection decreased by 80% in presence of AXL siRNA compared to absence.

USE - The method is useful for decreasing and treating infection of a host cell by a virus, such as HIV, influenza A or Ebola virus. Specifically, especially where the pathogen hijacks a lipid raft, the method is useful for decreasing infection of *Campylobacter jejuni*, *Vibrio cholerae* SV40, *Legionella pneumophila*, *Aeromonas hydrophila*, Echovirus 1, Echovirus 11, *Brucella* spp., *Clostridium* spp., Avian sarcoma and leukosis virus, FimH, *Escherichia coli*, *Streptococcus pyogenes*, Semliki forest virus, *Salmonella typhimurium*, *Bacillus anthracis*, Ecotropic mouse leukaemia virus, *Shigella flexneri*, *Bacillus thuringiensis*, HTLV-I, *Chlamydia* spp., *Helicobacter pylori*, HFV-I, *Mycobacterium* spp., *Listeria monocytogenes*, Ebola, Marburg, Measles, Herpes Simplex virus, influenza virus, or Epstein-Barr virus (claimed).

Dwg.0/6

FS CPI

FA AB

MC CPI: B04-E03; B04-E06; B04-E07; B04-F0200E; B04-G01; B04-N02; B04-N03; B04-P01A0E; B11-C08E; B12-K04A; B12-K04E; B14-A01; B14-A02; D05-H09; D05-H11; D05-H12D2; D05-H12D6; D05-H14B2; D05-H16A

TECH UPTX: 20040920

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Method: In decreasing infection of a host cell by a virus, the host protein or host nucleic acid is a T-cell receptor V beta chain; T-cell receptor V-D-J beta 2.1 chain; beta-chimerin; malic enzyme 1; hypothetical protein XP174419; sequence from chromosome 4q31.3-32; alpha satellite DNA; LOC253788; LOC219938; coagulation factor m (F3); LOC91759; similar to KOX4 (LOC131880); LOC166140; LOC222474; similar to Rho guanine nucleotide exchange factor 4, isoform a; APC-stimulated guanine nucleotide exchange factor (LOC221178); T-cell receptor beta; ribosomal protein L7A-like 4; v-src sarcoma (Schmidt-Ruppin A-2) viral oncogene homolog (avian) (SRC); KIAA0564; alpha satellite DNA; M96 protein; hypothetical protein similar to G proteins (LOC57826); LOC161005; osteoblast specific factor 2; *Canis familiaris* T-cell leukemia translocation-associated protein; aminomethyltransferase; dystroglycan; bassoon; LIM domain containing preferred translocation partner in lipoma; sequence between LOC253121 and hyaluronan synthase 2; testin 2, testin 3; protein tyrosine phosphatase, non-receptor type 1; sequence between LOC149360 and LOC253961; sequence between KIAA1560 and tectorin beta; cadherin related 23; myeloid/lymphoma or mixed lineage leukemia, translocated to 10; exportin 5; DNA polymerase eta (POLH); heterogeneous nuclear riboprotein C (C1/C2); alpha-endosulfine pseudogene; LOC128741; LOC222888; LOC138421; zinc finger protein 297B; sideroflexin 5; importin 9 (FLJ10402); T-cell receptor beta; similar to murine putative transcription factor ZNF131 (LOC135952); KIAA1259; MURR1; CCT4; FLJ40773; similar to ribosomal protein L24-like (LOC149360); polybromo 1; DNA damage

inducible transcript 3; KIAA1887; PDZ ; LIM domain 1 (elfin); LOC284803; PRO0097; FLJ31958; small inducible cytokine E, member 1 (endothelial monocyte-activating); E3 ubiquitin ligase (SMURF2); MGC40489; Rab9; PRO1617; retinoblastoma binding protein 1; region of chromosome 2q12; elongation factor for selenoprotein translation; Transcription factor SMIF (HSA275986); KIAA1026; trinucleotide repeat containing 5 (TNRC5); homogentisate 1,2-dioxygenase (HGD); region of chromosome Xq23-24; region of chromosome 4p15.3; similar to LWamide neuropeptide precursor protein (Hydractinia echinata) (LOC129883); region of chromosome 2q21; region of chromosome Xp1 1.4, including UPS9X; LOC221829 ; U3 small nuclear RNA; integral, beta 1 (ITGB1) ; acrosomal vesicle protein 1 (ACRV1) and CHK1 checkpoint homolog (CHEK1); prospero-related homeobox 1 (PROX1); FLJ20627 and FLJ12910; PIN2-interacting protein (PINX1) and SRY (sex-determining region Y)-box 7 (SOX7); LOC131920; region of chromosome 13q14; neurotrophic tyrosine kinase, receptor, type 3 (NTRK3); TERA protein and FLJ13224; LOC284260; POM (POM121 homolog) and ZP3 fusion (POMZP3); DEAD/H box polypeptide 8 (DDX8) and similar to ribosomal protein L29 (cell surface heparin binding protein HIP) (LOC284064); LOC345307 and UDP-N-acetyl-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 7 (GALNT7); Mus musculus 5S rRNA pseudogene (Rn5s-ps1); ribosomal protein L27a pseudogene (RPL27AP) and v-myb myeloblastosis viral oncogene homolog-like 2 (MYBL2); Down's syndrome cell adhesion molecule like 1 (DSCAML1); LOC148529; Huntingtin-associated protein interacting protein (HAPIP); LOC158525 and similar to RIKEN cDNA 1210001E11 (LOC347366); hypothetical protein FLJ12910; LOC350411; allograft inflammatory factor 1 (AIF1) and HLA-B associated transcript 2 (BAT2); C10orf7; LOC346658 and LOC340349; region of chromosome 12q21; LOC339248 and FLJ22659; SR rich protein DKFZp564B0769 and hypothetical protein MGC14793; FLJ10439; cytochrome P450, family 11, subfamily A, polypeptide 1 (CYP1 IAL) and sema domain, immunoglobulin domain (Ig) and GPI membrane anchor, (semaphoring) 7A; ribosomal protein S16 (RPS 16); hypothetical protein DKFZp434H0115 and ATP citrate lyase (ACLY); calnexin (CANX); protein tyrosine phosphatase, receptor type, K (PTPRK); cyclin M2 (CNNM2); or AXL receptor tyrosine kinase (AXL), and where interfering with the activity or expression of the one or more host proteins decreases infection of the host cell by the virus. The one or more host proteins is encoded by one or more host nucleic acids comprising, or having at least at least 90% identity to any target nucleic acid sequence associated with any of the 229 sequences (S1-S229), fully defined in the specification. The method comprises interfering with an activity or expression of more than one, or at least three of the host proteins. The virus is HIV-1 or HIV-2. The method comprises interfering with expression of one or more of the host nucleic acids. The virus is influenza A, and the host protein is a Canis familiaris T-cell leukemia translocation-associated protein, aminomethyltransferase; dystroglycan; bassoon; LJM domain containing preferred translocation partner in lipoma; sequence between LOC253121 and hyaluronan synthase 2; testin 2; testin 3; PTPN1 gene for protein tyrosine phosphatase, non-receptor type 1; sequence between LOC1493 60 and LOC253961; sequence between KIAA1560 and tectorin beta; cadherin related 23; malic enzyme 1; hypothetical protein XP174419; sequence from chromosome 4q31.3-32; Rab9, or a myeloid/lymphoma or mixed lineage leukemia, translocated to 10. The virus is Ebola, and the host protein is a exportin 5; DNA polymerase eta (POLH); heterogenous nuclear riboprotein C; alpha-endosulfine pseudogene; LOC128741; LOC222888; LOC138421; zinc finger protein 2977B; sideroflexin 5; importin; (FLJ10402); T-cell receptor beta; similar to murine putative transcription factor ZNF131 (LOC135952); KIAA1259; MURR1; CCT4; FLJ40773; ribosomal protein L24-like (LOC149360); testin 2; testin 3; polybromo 1; DNA damage inducible transcript 3; KIAA1887; PDZ; LIM domain 1 (elfin); LOC284803; PRO0097; FLJ31958; small inducible cytokine E, member 1 (endothelial monocyte-activating); E3 ubiquitin ligase; MGC40489; Rab9; PRO1617; retinoblastoma binding protein 1; region of chromosome 2q12; elongation factor for selenoprotein translation; Transcription factor SMIF

(HSA275986); KIAA1026; trinucleotide repeat containing 5 (TNRC5); homogentisate 1,2-dioxygenase (HGD); region of chromosome Xq23-24; region of chromosome 4p15.3; similar to LWamide neuropeptide precursor protein (Hydractinia echinata) (LOC129883); region of chromosome 2q21; region of chromosome Xp 11.4, including UPS9X; L(tm)C221829 ; U3 small nuclear RNA; integral, beta 1 (ITGB1); acrosomal vesicle protein 1 (ACRV1) and CHK1 checkpoint homolog (CHEK1); prospero-related homeobox 1 (PROX1); FLJ20627 and FLJ12910; PIN2-interacting protein (PINX1) and SRY (sex-determining region Y)-box 7 (SOX7); LOC131920; region of chromosome 13q14; neurotrophic tyrosine kinase, receptor, type 3 (NTRK3); TERA protein and FLJ13224; LOC284260; POM (POM121 homolog) and ZP3 fusion (POMZP3); DEAD/H box polypeptide 8 (DDX8) and similar to ribosomal protein L29 (cell surface heparin binding protein HIP) (LOC284064); LOC345307 and UDP-N-acetyl-D-galactosamine:polypeptide N-acetylgalactosaminyltransferase 7 (GALNT7); Mus musculus 5S rRNA pseudogene (Rn5s-ps1); ribosomal protein L27a pseudogene (RPL27AP) and v-myb myeloblastosis viral oncogene homolog-like 2 (MYBL2); Down's syndrome cell adhesion molecule like 1 (DSCAML1); LOC148529; Huntingtin-associated protein interacting protein (HAPIP); LOC1 58525 and similar to RIKEN cDNA 1210001E11 (LOC347366); hypothetical protein FLJ12910; LOC350411; allograft inflammatory factor 1 (AIF1) and HLA-B associated transcript 2 (BAT2); ClOorfJ; LOC346658 and LOC340349; region of chromosome 12q21; LOC339248 and FLJ22659; SR rich protein DKFZp564B0769 and hypothetical protein MGC14793; FLJ10439; cytochrome P450, family 11, subfamily A, polypeptide (CYP1 IAL) and sema domain, immunoglobulin domain (Ig) and GPI membrane anchor, (semaphoring) 7A; ribosomal protein S16 (RPS16); hypothetical protein DKFZp434H0115 and ATP citrate lyase (ACLY); calnexin (CANX); protein tyrosine phosphatase, receptor type, K (PTPRK); cyclin M2 (CNNM2); or AXL receptor tyrosine kinase.

Interfering with the activity of the one or more host proteins comprises decreasing an interaction of a viral protein and the one or more host proteins by disrupting or decreasing expression of the one or more host proteins. The viral protein comprises a virus and decreasing the interaction of the viral protein and the one or more host proteins decreases or inhibits infection of a host cell by the virus. Disrupting or decreasing expression of the host protein comprises disrupting or decreasing transcription of an mRNA encoding the host protein. Disrupting or decreasing transcription of the mRNA comprises inserting a transposon or insertional vector into a coding region of the nucleic acid encoding the host protein. Disrupting or decreasing the transcription of the mRNA comprises contacting the mRNA with an antisense RNA, RNAi, ribozyme, or siRNA that recognizes the mRNA. Interfering with the activity of the host protein comprises decreasing an interaction of a viral protein and the host protein by contacting the cell with an agent that decreases or inhibits the activity or expression of the host protein or that disrupts expression of the host protein. The host cell is present in a host subject and where contacting the cell with the agent comprises administering the agent to the subject. The host cell is a mammalian host cell.

Decreasing HIV, Ebola, or influenza A infection of a host cell comprises decreasing an interaction between a viral nucleic acid and a host nucleic acid by decreasing the integration of the viral nucleic acid into the host nucleic acid. The viral nucleic acid comprises a viral genome and the host nucleic acid comprises a host genome. This method alternatively comprises contacting the host cell with an anti-protein binding agent that selectively or specifically binds to a host protein encoded by any target sequence associated with S1-S229, where the anti-protein binding agent inhibits an interaction between the host protein and the HIV, Ebola, or influenza A virus. The host cell is present in a subject, and contacting the host cell with the anti-protein binding agent comprises administering the anti-protein binding agent to the subject. The anti-protein binding agent is an antibody or chemical compound.

Treating an HIV, Ebola, or influenza A viral infection in a host subject comprises administering to a subject having a viral infection an effective

amount of an agent that interferes with the interaction of a virus and host protein. The agent disrupts expression of the nucleic acid encoding the host protein. The agent is an antisense, ribozyme, or siRNA molecule that recognizes the nucleic acid sequence comprising at least 90% identity to any target sequence associated with S1-S229. The effective amount induces a prophylactic effect in the host, which inhibits infection of the host by a virus. The host was previously infected by a virus and the effective amount induces a therapeutic effect in the host.

Determining resistance or susceptibility to viral infection in a subject comprises comparing a first nucleic acid sequence of a subject to a second nucleic acid sequence comprising any target sequence associated with S1-S229, where a higher similarity between the first and second nucleic acid sequence indicates the subject is more susceptible to viral infection, and where a lesser similarity between the first and second nucleic acid sequence indicates the subject is more resistant to viral infection. The first nucleic acid sequence is obtained from a biological sample of the subject. The first nucleic acid sequence comprises a plurality of nucleic acid sequences, where each nucleic acid sequence is obtained from a different subject. This method further comprises determining a polymorphic variation within a population.

Identifying a compound that decreases binding of a viral protein to a host protein and decreases viral infection comprises contacting the host protein with the viral protein and a test compound, wherein the host protein is any of the protein listed in the specification, and the viral protein is an HIV, Ebola, or influenza A protein; and determining whether binding of the viral protein to the host protein is decreased in the presence of the test compound, the decrease in binding being an indication that the test compound decreases the binding of viral protein to the target protein, and decreases viral infection. The viral protein comprises a virus. The viral protein is a viral envelope protein. The viral protein is an HIV protein. This method comprises expressing the host protein in a cell, and contacting the host protein with the viral protein and a test compound comprises exposing the cell to the viral protein and the test compound. The host protein or the viral protein comprises a label, and determining whether binding is decreased comprises detecting an amount of label present.

Decreasing infection of a host cell by a pathogen comprises interfering with an activity or expression of a Rab9 in the host cell, where interfering with Rab9 activity or expression decreases infection of the host cell by the pathogen. The pathogen hijacks a lipid raft. The pathogen is a *Campylobacter jejuni*, *Vibrio cholerae* SV40, *Legionella pneumophila*, *Aeromonas hydrophila*, Echovirus 1, Echovirus 11, *Brucella* spp., *Clostridium* spp., Avian sarcoma and leukosis virus, FimH, *Escherichia coli*, *Streptococcus pyogenes*, Semliki forest virus, *Salmonella typhimurium*, *Bacillus anthracis*, Ecotropic mouse leukemia virus, *Shigella flexneri*, *Bacillus thuringiensis*, HTLV-I, *Chlamydia* spp., *Helicobacter pylori*, HFV-I, *Mycobacterium* spp., *Listeria monocytogenes*, Ebola, Marburg, Measles, Herpes Simplex virus, influenza virus, or Epstein-Barr virus. Interfering with expression of Rab9 comprises disrupting or decreasing transcription of an mRNA encoding the Rab9 protein. Disrupting or decreasing the transcription of the mRNA comprises contacting the mRNA with an antisense RNA, ribozyme, or siRNA that recognizes the mRNA. The host cell is present in a subject, and contacting the mRNA with an antisense RNA, ribozyme, or siRNA that recognizes the mRNA comprises administering the antisense RNA, ribozyme, or siRNA to the subject.

L46 ANSWER 2 OF 7 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN

AN 2004-315601 [29] WPIX

DNN N2004-251489 DNC C2004-119632

TI Identifying protein-protein interactions, useful e.g. in drug development, comprises introducing into cells one or more prey proteins labeled with an epitope tag and one or more bait proteins labeled with a detectable substance.

DC B04 D16 S03  
 IN BARRIOS-RODILES, M; WRANA, J  
 PA (MOUN) MOUNT SINAI HOSPITAL  
 CYC 105  
 PI WO 2004023146 A2 20040318 (200429)\* EN 53 G01N033-68  
 RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE LS  
 LU MC MW MZ NL OA PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW  
 W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK  
 DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR  
 KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NI NO NZ OM PG PH  
 PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG US UZ VC  
 VN YU ZA ZM ZW  
 AU 2003264211 A1 20040329 (200459) G01N033-68  
 ADT WO 2004023146 A2 WO 2003-CA1354 20030905; AU 2003264211 A1 AU 2003-264211  
 20030905  
 FDT AU 2003264211 A1 Based on WO 2004023146  
 PRAI US 2002-408922P 20020906  
 IC ICM G01N033-68  
 AB WO2004023146 A UPAB: 20040505  
 NOVELTY - Identifying protein-protein interactions comprising prey  
 proteins interacting with one or more bait comprises introducing one or  
 more prey proteins in labeled with an epitope tag and one or more bait  
 protein in cells labeled with a detectable substance.  
 DETAILED DESCRIPTION - Identifying protein-protein interactions  
 comprising prey proteins interacting with one or more bait comprises:  
 (a) introducing one or more prey proteins in cells, where a prey is  
 labeled with an epitope tag permitting separation of the prey protein from  
 other proteins in the cells;  
 (b) introducing one or more bait protein in cells, where a bait  
 protein is labeled with a detectable substance permitting detection of the  
 bait protein and protein-protein interactions comprising a prey protein  
 and the bait protein;  
 (c) inducing formation of protein-protein interactions between a prey  
 and bait protein; and  
 (d) assaying for protein-protein interactions comprising a prey  
 protein and bait protein by detecting the detectable substance.  
 INDEPENDENT CLAIMS are also included for:  
 (1) quantitating protein-protein interactions;  
 (2) determining an interactome for one or more bait protein;  
 (3) determining the functions of gene product;  
 (4) systematically and quantitatively analyzing protein-protein  
 interactions in cell signaling;  
 (5) determining the changes in an interactome of mitotic kinase  
 during cell cycle progression;  
 (6) analyzing protein-protein interactions in different cell types;  
 (7) assaying for changes in protein-protein interactions in response  
 to intracellular and extracellular factors;  
 (8) identifying a potential modulator of signal transduction  
 activity; and  
 (9) an agent, modulator or inhibitor identified by a method of (8).  
 ACTIVITY - Antiinflammatory; Cytostatic.  
 No biological data given.  
 MECHANISM OF ACTION - None Given.  
 USE - The method and kits are useful in identifying, quantifying and  
 analyzing protein-protein interactions. The method is useful in  
 determining a disease or condition associated with a test protein,  
 monitoring the course of therapy, conducting a drug discovery business and  
 in detecting mutations in cellular proteins. The pharmaceutical  
 composition is useful in treating and preventing a disease or condition  
 associated with an abnormality in a signal transduction pathway, e.g.  
 fibrosis, inflammation or cancer.  
 Dwg.0/3  
 FS CPI EPI

FA AB  
MC CPI: B04-G01; B04-G21; B04-G22; B11-C07A; B11-C08E1; B11-C08F4; B12-K04A;  
B12-K04E; B14-C03; B14-H01; D05-H08; D05-H09; D05-H11  
EPI: S03-E14H4  
TECH UPTX: 20040505

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Method: Quantitating protein-protein interactions comprises the steps (a)-(c) of the method above and quantitating the protein-protein interactions comprising a prey and bait protein. Alternatively, the method comprises expressing one or more prey protein and bait protein in cells, obtaining a lysate of the cells and assaying an aliquot of the lysate to measure total expression of the epitope tag and detectable substance, assaying a second aliquot of the lysate to measure the amount of a detectable substance that coprecipitates with an epitope tagged prey protein and comparing the amount measured in (b) and (c) to quantitate the protein-protein interaction. The cells are subjected to an extracellular or intracellular signal after expressing the proteins.

Determining an interactome for one or more bait protein comprises preparing recombinant cells each expressing one or more bait protein and one or more prey protein from a variegated population of prey proteins, inducing formation of protein-protein interactions between a prey and bait protein in the cells and identifying protein-protein interactions comprising a prey and bait protein. Determining the functions of gene product comprises defining an interactome of the gene product using the method of (2) and determining the function of the gene product based on the structure and/or function of prey proteins that interact with the gene product in the interactome. Systematically and quantitatively analyzing protein-protein interactions in cell signaling comprises the steps of a method of identifying protein-protein interactions and comparing the types of and quantitating protein-protein interactions at the different time points.

Determining the changes in an interactome of mitotic kinase during cell cycle progression comprises introducing into the cells one or more prey protein labeled with an epitope tag permitting separation of the prey protein from other proteins in the cells and one or more mitotic kinase labeled with a detectable substance permitting identification of mitotic kinase and protein-protein interactions comprising the mitotic kinase and a prey protein, assaying for protein-protein interactions comprising a prey and mitotic kinase at different time points and comparing the types and kind of protein-protein interactions at different time points.

Analyzing protein-protein interactions in different cell types comprises introducing into first and second cells one or more prey protein and one or more bait protein, inducing cell signaling in first and second cells forming protein-protein interactions comprising a prey and bait protein and comparing the protein-protein interaction identified in the first and second cells. The first cells are from a subject with disease and the second cells are normal cells.

Assaying for changes in protein-protein interactions in response to intracellular and extracellular factors comprises introducing one or more prey proteins and one or more bait proteins in cells, inducing formation of protein-protein interactions between a prey and bait protein, introducing an intracellular or extracellular factor, assaying protein-protein interaction comprising a prey and bait protein and comparing the assayed protein-proteins interactions in the absence of intracellular or extracellular factors.

Identifying a potential modulator of signal transduction activity comprises introducing one or more prey protein and one or more bait proteins in cells, introducing a test agent in the cell, inducing formation of protein-protein between a prey and bait protein, assaying protein-protein interaction comprising a prey and bait protein and comparing the assayed protein-proteins interactions in the absence of a test agent to determine the effect of the agent on the protein-protein interactions, where a change in the protein-protein interactions indicates

that the test agent is a potential modulator. An increase and decrease in the protein-protein interactions respectively indicates that the agent is an agonist or antagonist. The cells are mammalian cells. One or more bait and prey proteins is introduced or expressed in the cells. The detectable substance is an enzyme, radioisotope, fluorescent label, luminescent label, preferably an enzymatic label, i.e. luciferase, specifically Renilla luciferase. The epitope tag is FLAG, hemagglutinin, His6 or an Ig sequence. The prey protein comprises a protein sequence obtained from genomic DNA sequences or random sequences or a library of protein sequences. The bait protein is a functional domain of a protein involved in signal transduction. The bait protein is a protein of the TGFbeta proteome, Wnt/Wingless pathway, Sak/Polo pathway or a receptor tyrosinase kinase pathway. The bait protein is a Smad protein, SARA family protein, Smad-interacting protein, TGF beta receptor, TGF beta receptor interacting protein, SMURF, BMP receptor, APC, beta-catenin, axin, disheveled, GSK-3 beta, TCFs1-4, Sak, Plks, EGF, FGF, PDGF or NGF. Protein-protein interactions are assayed by purifying prey protein and complexes comprising the prey protein based on epitope tag and co-purifying the protein-protein interactions comprising the prey and bait protein by detecting the detectable substance. The prey and bait protein and complexes are purified by immunoprecipitation with an antibody specific for the epitope tag.

ABEX UPTX: 20040505

WIDER DISCLOSURE - (1) Constructing a protein linkage map for a proteome or interactome; and  
(2) an integrated modular system for performing the methods.

ADMINISTRATION - Administration is by oral, subcutaneous, intravenous, intraperitoneal, intranasal, enteral, topical, sublingual, intramuscular, intraarterial, intramedullary intrathecal, inhalation, transdermal or rectal means. No dosage given.

EXAMPLE - No relevant example given.

L46 ANSWER 3 OF 7 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN

AN 2004-247973 [23] WPIX

DNN N2004-196735 DNC C2004-096855

TI Diagnosing glioma by detecting expression product of any one of 255 genes, glioma endothelial markers, in brain tissue sample suspected of being neoplastic, and comparing the expression with expression in normal brain tissue sample.

DC B04 D16 S03

IN COOK, B P; LATTEA, J; MADDEN, S I; WALTER, K; WANG, C J

PA (GENZ) GENZYME CORP; (UYJO) UNIV JOHNS HOPKINS

CYC 105

PI WO 2004016758 A2 20040226 (200423)\* EN 114 C12N000-00

RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR HU IE IT KE LS  
LU MC MW MZ NL OA PT RO SD SE SI SK SL SZ TR TZ UG ZM ZW

W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK  
DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR  
KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NI NO NZ OM PG PH  
PL PT RO RU SC SD SE SG SK SL SY TJ TM TN TR TT TZ UA UG US UZ VC  
VN YU ZA ZM ZW

AU 2003262717 A1 20040303 (200457) C12N000-00

ADT WO 2004016758 A2 WO 2003-US25614 20030815; AU 2003262717 A1 AU 2003-262717 20030815

FDT AU 2003262717 A1 Based on WO 2004016758

PRAI US 2003-458978P 20030401; US 2002-403390P 20020815

IC ICM C12N000-00

AB WO2004016758 A UPAB: 20040405

NOVELTY - Aiding in diagnosis of glioma involves detecting expression product of at least one gene (I) in first brain tissue sample (T) suspected of being neoplastic, where (I) is chosen from any one of 255

genes (glioma endothelial markers (GEMs)) as given in specification, and comparing expression of (I) in (T) with expression of (I) in second normal brain tissue sample (R), where increased expression of (I) in (T) relative to (R), identifies (T) as likely to be neoplastic.

DETAILED DESCRIPTION - Aiding (M1) in diagnosing glioma involves detecting an expression product of at least one gene (I) in a first brain tissue sample suspected of being neoplastic, where (I) is chosen from any one of 255 genes (glioma endothelial markers (GEMs)) as given in specification, e.g., signal sequence receptor, delta (translocon-associated protein delta ); DC2 protein; KIAA0404 protein; symplekin; Huntingtin interacting protein I; plasmalemma vesicle associated protein; KIAA0726 gene product; latexin protein; transforming growth factor, beta 1; hypothetical protein FLJ22215; Rag C protein; hypothetical protein FLJ23471; N-myristoyltransferase 1; hypothetical protein dJ1181N3.1; ribosomal protein L27; secreted protein, acidic, cysteine-rich (osteonectin); Hs 111988; Hs 112238; laminin, alpha 5; protective protein for beta -galactosidase (galactosialidosis); Melanoma associated gene; E3 ubiquitin ligase **SMURF1**; collagen, type IV, alpha 1; insulin-like growth factor binding protein 7; gene predicted from cDNA with a complete coding sequence; Thy-1 cell surface antigen; Hs127824; GTP binding protein 2; Homo sapiens mRNA; cDNA DKFZp586D0918 (from clone DKFZp586D0918); cutaneous T-cell lymphoma-associated tumor antigen se20-4; differentially expressed nucleolar TGF- beta 1 target protein (DENTT); dysferlin, limb girdle muscular dystrophy 2B (autosomal recessive); smoothelin; integrin, alpha 5 (fibronectin receptor, alpha polypeptide); putative translation initiation factor; retinoic acid induced 14; matrix metalloproteinase 9 (gelatinase B, 95 kD gelatinase, 92 kD type IV collagenase); Lutheran blood group (Auberger b antigen included); stanniocalcin 2; nuclear factor (erythroid-derived 2)-like 2; protein tyrosine phosphatase, non-receptor type 1; etc; and comparing expression of (I) in (T) with expression of (I) in second normal brain tissue sample (R), where increased expression of (I) in (T) relative to (R), identifies (T) as likely to be neoplastic. The method optionally involves detecting mRNA of at least one gene in (T), where the at least one gene is identified by a tag which has a sequence of AAACCATTCT, AAGGCAGGGA, ACACAGCAAG, AGCTGGAGTC, AGCTGGCACC, ATAAATGAGG, CAAGCACCCC, CACTACCCAC, CACTACTCAC, CCCACCTCCA, CCCGCCTCTT, CCTCAGATGT, CGCTACTCAC, CTAAGACCTC, CTAAGACTTC, GAGTGGGTGC, GGGACAGCTG, GGGTTGGCTT, GTAAGTGATC, GTAAGTGATC, GTAGGGGTAA, TAACCACTGC, TACTGCTCGG, TCAGGCTGAA, TCCATACACC, TCCTTTTAAA, TGATTAAGGT, TGGTATCACA, TGGTGTATGC, TGTCCTGGG, TGTGGGAGGC, or TTTAACGGCC (S1-S32), and comparing expression of the at least one gene in (T) with expression of the gene in (R), where increased expression of the gene in (T) relative to (R) identifies (T) as likely to be neoplastic.

INDEPENDENT CLAIMS are also included for the following:

(1) treating (M2) glioma involves contacting cells of the glioma with an antibody that specifically binds to a extracellular epitope of protein chosen from plasmalemma vesicle associated protein; KIAA0726 gene product; osteonectin; laminin, alpha 5; collagen, type IV, alpha 1; insulin-like growth factor binding protein 7; Thy-1 cell surface antigen; dysferlin, limb girdle muscular dystrophy 2B; integrin, alpha 5; matrix metalloproteinase 9; Lutheran blood group, integrin, alpha 10, collagen, type VI alpha 2; glioma endothelial marker 1 precursor; translocase of inner mitochondrial membrane 17 homolog A; heparan sulfate proteoglycan 2; annexin A2; matrixmetalloproteinase 10; G protein-coupled receptor; matrix metalloproteinase 14; solute carrier family 29, member 1; CD59 antigen p18-20; KIAA 1870 protein; plexin B2; lectin; integrin beta 4 binding protein; acetyl low density lipoprotein (LDL) receptor; laminin, gamma 3; macrophage migration inhibitory factor; gap junction protein, alpha 1, 43 kD; aquaporin 1; protease, serine, 11; collagen, type IV, alpha 2; apolipoprotein D; plasminogen activator; urokinase; insulin-like growth factor binding protein 3; regulator of G-protein signaling 12; prosaposin; etc.;

(2) identifying (M3) a test compound as potential anticancer or

antiglioma drug involves contacting a test compound with the cell which expresses (I), monitoring an expression product of the at least one gene and identifying test compound as a potential anticancer drug if it decreases the expression of at least one gene;

(3) identifying (M4) a test compound as potential anticancer or anti glioma drug involves contacting a test compound with the cell which expresses mRNA of at least one gene identified by a tag as described above, monitoring mRNA of the gene, and identifying the test compound as a potential anticancer drug if it decreases the expression of at least one gene; and

(4) inducing (M5) an immune response to glioma involves administering to a mammal, a protein or (I).

ACTIVITY - Cytostatic.

MECHANISM OF ACTION - Triggers immune destruction of glioma cells; Immune response inducer. No supporting data is given.

USE - (M1) is useful for aiding in diagnosing glioma. (M2) is useful for treating multi-drug sensitive glioma in a human. (M5) is useful for inducing an immune response to a glioma in a mammal having glioma or in a mammal who has had a glioma surgically removed (claimed).

Dwg.0/0

FS CPI EPI

FA AB; DCN

MC CPI: B04-E03E; B04-E03F; B04-E12; B04-F02; B04-F02A; B04-G01; B04-H01; B04-H06F; B04-L01; B04-N04; B04-N06; B11-C07A; B11-C07A3; B11-C08E3; B11-C08E5; B11-C08E6; B12-K04A1; B12-K04E; B12-K04F; B14-H01; D05-H08; D05-H09; D05-H11; D05-H12A

EPI: S03-E14H4

TECH UPTX: 20040405

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Method: Increased expression of (I) in (T) relative to (R) is at least two-fold higher, preferably at least 10-fold higher. The expression product is RNA or protein, where the protein is detected using Western blot or by an immunoassay or immunohistochemical assay, and the RNA is detected using serial analysis of gene expression (SAGE), or by using hybridization to microarray. (T) and (R) are from a human, preferably same human. Detecting In (M2), the antibody is conjugated to a diagnostic or therapeutic agent, e.g., chemotherapeutic agent, cytotoxin, a nonradioactive label, or radioactive compound. In (M3) or (M4), the test compound is contacted with the human glioma cell, where the cell overexpresses at least one gene relative to normal cell of the same tissue. The expression product is a protein or RNA. The method involves monitoring expression of at least two of the genes, preferably four of the genes. The test compound is identified if the decrease in expression is at least 50%, preferably 90%. The test compound is identified as an anti glioma drug. (M5) further involves administering an immune adjuvant to the mammal.

ABEX UPTX: 20040405

WIDER DISCLOSURE - Use of glioma endothelial markers for identifying endothelial cells, and for stimulating the growth of vasculature, such as for wound healing, or to circumvent a blocked vessel, is also disclosed.

ADMINISTRATION - The nucleic acid is administered intramuscularly (claimed). The proteins and nucleic acids are administered by parenteral, intravenous, intraperitoneal, topical, intranasal, intrarectal or intrabronchial route.

EXAMPLE - Five separate brain tissue samples were resected and immediately subjected to endothelial cell isolation. Briefly, samples were surgically excised and submerged in Dulbeccos' modified Eagle's medium (DMEM). The samples were minced into 2 cm<sup>3</sup> and subjected to tissue digestion with a collagenase cocktail. Samples were mixed at 37 degrees C until dissolved. Cells were spun down and washed two times with phosphate buffered saline/bovine serum albumin (PBS/BSA) and filtered through successive nylon mesh filters of 250, 100 and 40 microns. Samples were resuspended in PBS/BSA and applied to a 30% Percoll gradient centrifugation. Five ml off

the top of the Percoll gradient was diluted in 50 ml DMEM and cells pelleted, washed with PBS and resuspended in 3 ml PBS/BSA. Cells were filtered through falcon blue top filter tubes, spun down and resuspended in 1 ml PBS/BSA. 100 microl of prewashed anti-CD45 magnetic beads were added and the solution allowed to gently mix for ten minutes. Bead-bound cells were discarded and the supernatant transferred to a fresh microcentrifuge tube. 10 microl of P1H12 mAB (1:100) (Brain N1, T1, and T2 samples) or UEA-I lectin (Brain N2 and T3 samples) was added and the samples were mixed gently at 4 degrees C for 45 minutes. Cells were pelleted and washed 3 times in PBS/BSA and resuspended in 500 microl PBS/BSA. Prewashed goat anti-mouse M450 Dynabeads were added to each tube and allowed to mix for 15 minutes at 4 degrees C. Bead-bound cells were washed 8 times with PBS/BSA and resuspended in a final volume of 500 microl PBS. Cells were counted and frozen at -70 degrees C prior to RNA extraction. RNA was isolated from the selected cells and initially subjected to reverse transcriptase polymerase chain reaction (RT-PCR) analysis to determine the relative abundance of specific, known endothelial cell markers. The microserial analysis of gene expression (SAGE) protocol was used to generate high-quality longSAGE libraries employing the tagging enzyme MmeI instead of BsmFI. 21 base tags were defined by capillary sequencing using a combination of an ABI 3700 and ABI 3100. Long SAGE tags derived from the brain endothelial samples were reduced to short tags to allow for the integration of colon endothelial SAGE data. Aggregate short tags were derived from the long tags. Any short tag counts that had more than one corresponding long tag representative were summed and the counts represented as one short tag. Both sequencing errors and legitimate long tag derivatives contribute to the generation of multiple long tags. For transcript and genome mapping, differential long tags were employed. Differential gene expression was evaluated as follows: For the two normal brain samples, either the maximum or minimum value was used for determining tumor/normal and normal/tumor ratios, respectively. For the three brain tumor samples, the median value was used for the tumor/normal whereas the maximum value was used for the normal/tumor ratios. A two parameter family of beta distributions was used to assess the probability of observing two fold differences in the observed SAGE tag abundances. 255 human genes were identified that were expressed at significantly higher levels in brain tumor endothelium than in normal brain endothelium. These markers were named as glioma endothelial markers (GEMs).

L46 ANSWER 4 OF 7 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN  
 AN 2003-430236 [40] WPIX  
 DNN N2003-343514 DNC C2003-113665  
 TI Treatment of bone defect conditions involves use of compound or protocol,  
 which is inhibitory to ubiquitin ligases.  
 DC B04 S03  
 IN CHEN, D; GARRETT, I R; MUNDY, G R; ROSSINI, G; ZHAO, M  
 PA (CHEN-I) CHEN D; (GARR-I) GARRETT I R; (MUND-I) MUNDY G R; (ROSS-I)  
 ROSSINI G; (ZHAO-I) ZHAO M; (OSTE-N) OSTEOSCREEN INC  
 CYC 101  
 PI WO 2003030924 A1 20030417 (200340)\* EN 19 A61K038-00  
 RW: AT BE BG CH CY CZ DE DK EA EE ES FI FR GB GH GM GR IE IT KE LS LU  
 MC MW MZ NL OA PT SD SE SK SL SZ TR TZ UG ZM ZW  
 W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK  
 DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR  
 KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT  
 RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG UZ VC VN YU ZA ZM  
 ZW  
 US 2003092603 A1 20030515 (200340) A61K031-00  
 ADT WO 2003030924 A1 WO 2002-US33615 20021009; US 2003092603 A1 Provisional US  
 2001-328300P 20011009, Provisional US 2002-346742P 20020107, US  
 2002-268374 20021009  
 PRAI US 2002-346742P 20020107; US 2001-328300P 20011009;

US 2002-268374 20021009

IC ICM A61K031-00; A61K038-00

ICS A61K039-00; C12Q001-68; G01N033-567

AB WO2003030924 A UPAB: 20030624

NOVELTY - Treatment of bone defect conditions involves administering to a subject, a protocol or a compound, which is inhibitory to beta -TrCP, Smad ubiquitin regulatory factor-1 (**Smurf1**) or **Smurf2**.

DETAILED DESCRIPTION - An INDEPENDENT CLAIM is included for identification of compound or protocol, which enhances bone formation and/or osteoblast differentiation involving assessing the ability of a candidate compound or protocol to inhibit activity of beta -TrCP, **Smurf1** or **Smurf2**.

ACTIVITY - Osteopathic; Antiarthritic; Cytostatic; Vulnerary; Antiinflammatory; Periodontal.

Test details are described but no suitable results are given.

MECHANISM OF ACTION - Bone growth stimulator or bone formation modulator; beta -TrCP, **Smurf1** and **Smurf2** inhibitor.

USE - For the treatment of bone defect conditions (claimed) (e.g. osteoporosis including age related osteoporosis, post-menopausal osteoporosis, glucocorticoid-induced osteoporosis or disuse osteoporosis and arthritis); for repair of congenital trauma-induced or surgical resection of bone e.g. cancer treatment, and in cosmetic surgery; for tooth repair; for treating cartilage defects or disorders; and in wound healing or tissue repair. Also useful for the treatment of growth deficiencies, periodontal diseases and defects.

ADVANTAGE - The compound or the protocol enhances bone formation and osteoblast differentiation. The compounds repair bone defects and deficiencies occurring in closed, open and non-union fractures, in closed and open fracture reduction, promotes bone healing in plastic surgery, stimulates bone in growth into non-cemented prosthetic joints and dental implants, elevates the peak bone mass in pre-menopausal women, increases bone formation during distraction osteogenesis. Therefore, the compounds clinically increase healing rates in fracture repair, reverse bone loss in osteoporosis, reverse cartilage defects, prevent or delay onset of osteoporosis, stimulate or augment bone formation in fracture non-unions and distract osteogenesis, increase bone growth in prosthetic device and repair dental defects. The compound stimulates growth of bone-forming cell precursors either in vitro or ex vivo and modifies a target tissue or organ environment so as to attract bone-forming cells to an environment in need of such cells, particularly the compounds stimulate a cell population containing marrow mesenchymal cell thus increasing the number of osteogenic cells in that cell population.

Dwg.0/6

FS CPI EPI

FA AB; DCN

MC CPI: B04-C01A; B04-H01; B04-L08; B07-D03; B14-C09; B14-D10; B14-E11; B14-H01; B14-N01; B14-N06B; B14-N17B

EPI: S03-E14H

ABEX UPTX: 20030624

ADMINISTRATION - The compounds are administered in a daily dosage of 0.1 - 1000 (preferably 1 - 200) mg/kg parenterally (including intravenously, subcutaneously, intramuscularly, intraperitoneally, intranasally or transdermally), enterally (including orally or rectally) or topically. The parenteral dosage is 20 - 100% of the oral dosage.

EXAMPLE - No relevant example given.

L46 ANSWER 5 OF 7 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN

AN 2003-120443 [11] WPIX

DNC C2003-031029

TI New breast cancer-associated (BCA) genes and polypeptides, useful for preventing, treating, diagnosing or staging breast cancer, or other BCA-related disorders, e.g. prostate cancer, sarcoma, Ewing's tumor, leukemia or lymphomas.

DC B04 D16  
 IN SETH, A  
 PA (SUNN-N) SUNNYBROOK & WOMEN'S COLLEGE HEALTH SCI  
 CYC 101  
 PI WO 2002087507 A2 20021107 (200311)\* EN 195 A61K000-00  
 RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ  
 NL OA PT SD SE SL SZ TR TZ UG ZM ZW  
 W: AE AG AL AM AT AU AZ BA BB BG BR BY BZ CA CH CN CO CR CU CZ DE DK  
 DM DZ EC EE ES FI GB GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR  
 KZ LC LK LR LS LT LU LV MA MD MG MK MN MW MX MZ NO NZ OM PH PL PT  
 RO RU SD SE SG SI SK SL TJ TM TN TR TT TZ UA UG US UZ VN YU ZA ZM  
 ZW  
 EP 1399460 A2 20040324 (200421) EN C07H021-02  
 R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT  
 RO SE SI TR  
 AU 2002311869 A1 20021111 (200433) A61K000-00  
 ADT WO 2002087507 A2 WO 2002-US13584 20020429; EP 1399460 A2 EP 2002-739203  
 20020429, WO 2002-US13584 20020429; AU 2002311869 A1 AU 2002-311869  
 20020429  
 FDT EP 1399460 A2 Based on WO 2002087507; AU 2002311869 A1 Based on WO  
 2002087507  
 PRAI US 2001-287170P 20010427  
 IC ICM A61K000-00; C07H021-02  
 ICS C12N005-12; C12N015-63; C12P021-06  
 AB WO 200287507 A UPAB: 20030214  
 NOVELTY - An isolated polynucleotide, which comprises a breast  
 cancer-associated (BCA) gene or a polynucleotide sequence encoding a  
 chimeric protein, is new. The polynucleotide consists of the human BCA1,  
 BCA2, BCA3, BCA4, BCA5, BCA6 or BCA7 gene.  
 DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the  
 following:  
 (1) A human BCA polypeptide encoded by the polynucleotide;  
 (2) Fragments of the human BCA polypeptide;  
 (3) A complex comprising BCA1 polypeptide and a binding partner  
 consisting of a gene product of AIP4, Smurf2, polyubiquitin UbC,  
 DUT, EPS15, ZBRK1, chromosome 10 open reading frame 5, AMSH, PLAT, TOM1L2,  
 FLJ11626, clone 155, VIM, INVS, clone 287, clone 292 or POLR2J;  
 (4) An antibody that immunospecifically binds to a human BCA1, BCA2,  
 BCA3, BCA4, BCA5, BCA6 or BCA7 polypeptide;  
 (5) An expression vector comprising the human BCA polynucleotide;  
 (6) A cell comprising a recombinant human BCA1, BCA2, BCA3, BCA4,  
 BCA5, BCA6 or BCA7 polynucleotide;  
 (7) A transgenic non-human animal comprising a transgene that  
 comprising the human BCA1, BCA2, BCA3, BCA4, BCA5, BCA6 or BCA7  
 polynucleotide;  
 (8) Diagnosing (M1) and staging a BCA-related disorder in a subject;  
 (9) Identifying (M2) an analyte that binds to the BCA polypeptide;  
 (10) Identifying (M3) a protein that binds the BCA polypeptide;  
 (11) Identifying (M4) an analyte that binds a complex comprising the  
 BCA polynucleotide or polypeptide;  
 (12) Identifying (M5) an analyte that inhibits formation of a complex  
 comprising the BCA polynucleotide or polypeptide;  
 (13) Identifying (M6) an inhibitor of growth of a breast cancer cell;  
 and  
 (14) A kit comprising:  
 (a) a first container with a purified BCA nucleic acid, BCA  
 polypeptide, BCA agonist, or BCA antagonist; and  
 (b) a second container with a molecule that binds to the BCA nucleic  
 acid, BCA polypeptide, BCA agonist or BCA antagonist when bound to an  
 analyte in a biological sample.  
 ACTIVITY - Cytostatic; Immunostimulant; Antiallergic; Osteopathic;  
 Anabolic.  
 No biological data given.

## MECHANISM OF ACTION - Gene Therapy; Vaccine.

No biological data given.

USE - The BCA polynucleotide, BCA polypeptide, anti-BCA polypeptide antibody, expression vector or antisense BCA polynucleotide is useful for preventing or treating breast cancer. These are also useful for diagnosing or staging a BCA-related disorder such as breast cancer (all claimed). Other BCA-related disorders that may be treated with the BCA polynucleotide or polypeptide or antibody are allergy, bone disease, eating disorder, infectious disease, ovarian cancer, prostate cancer, skin cancer or brain cancer, malignant or non-malignant tumors, sarcoma, Ewing's tumor, leukemia, lymphomas, or polycythemia vera. The BCA polynucleotide and polypeptide are also useful in forensic biology, diagnostic assays, prognostic assays or pharmacogenomics, or for monitoring clinical trials.

Dwg.0/22

FS CPI

FA AB; DCN

MC CPI: B04-C01A; B04-C01G; B04-E01; B04-F0100E; B04-G01; B04-G05; B04-N02A0E; B04-P0100E; B11-C07A; B11-C08E; B12-K04A1; B12-K04E; B14-E11; B14-G01; B14-G02A; B14-H01; B14-N01; B14-S03; B14-S11C; D05-C12; D05-H09; D05-H11; D05-H12A; D05-H12B1; D05-H12D; D05-H12E; D05-H14B; D05-H16A; D05-H17A6; D05-H17B6

TECH UPTX: 20030214

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Polynucleotide: The BCA polynucleotide comprises:

(a) a nucleotide sequence of the human BCA3 gene, which comprises 1319 bp fully defined in the specification;

(b) a nucleotide sequence that encodes a human BCA3 polypeptide;

(c) a nucleotide sequence that encodes the BCA3 polypeptide having 210 amino acids fully defined in the specification, or having the following sequence: Gly-Gly-Pro-Gly-Gly;

(d) at least 12 consecutive bases of the human BCA3 gene, where the polynucleotide is not F29989, BG754249, BG654786, AU146189, AU145473, AV729000, AV725974, BE349302, BE205860, AW406755, AW339687, AI635272, AI365988, BM469324, BM558580, AW510839, BF337353, AA640772, AL599210, AL571890, BF913170, BE149796, BG681808, AA478355, BE304890, BI058894, BM042507, BG773327, AA521399, AA521323, AI873852, BI030630, BI023028, BG819532, BE909262, BE293845, BE293802, AW675725, AW193295, F19258, AI358229, AA478297, BG566176, AJ400877, NM 020642 Nor BM449949; or

(e) a nucleotide sequence that is a complement of (d).

The polynucleotide comprises a nucleotide sequence of 640 bases in length that hybridizes under highly stringent conditions to:

(a) a nucleotide sequence complementary to the coding region of the human BCA3 gene; or

(b) the nucleotide sequence of a human BCA3 mRNA.

The isolated polynucleotide comprises a nucleotide sequence encoding a fragment of the human BCA3 protein, where the fragment displays one or more functional activities. The isolated polynucleotide comprises a BCA1 nucleotide sequence, which comprises residues 1-2659, 1-2500, 1-2000, 1-1500, 1-1000, 1-500, 1-124, 2516-2659, 2500-2659, 2000-2659, 1500-2659, 1000-2659, 500-2659, 124-2659, 363-377, or 551-674 of a 2659-bp sequence fully defined in the specification, or a 123-bp sequence or 19-bp sequence fully defined in the specification. The isolated polynucleotide also comprises a BCA2 nucleotide sequence consisting of residues 1-2176, 1-2000, 1-1500, 1-1000, 1-500, 1-100, 2000-2176, 1500-2176, 1000-2176, 500-2176, 100-2176, 768-782 or 980-1052 of a 2177-bp sequence fully defined in the specification, or a sequence having 15, 20, 15, 15 or 300 bp fully defined in the specification.

The polynucleotide also comprises a nucleotide sequence of at least 12 consecutive bases encoding a portion of a domain of:

(a) a human BCA1 polynucleotide or polypeptide, where the domain is RING H2, finger, PY motif, glycosylation site, phosphorylation site, SH2-binding motif, open-reading frame, exon 1, exon 2, exon 3, intron 1,

intron 2, 5' untranslated region, or 3' untranslated region;

(b) a human BCA2 polynucleotide or polypeptide, where the domain is RING H2, NPXXY motif, PXXP motif, zinc finger, glycosylation site, phosphorylation site, SH3-binding motif, open-reading frame, exon 1, exon 2, exon 3, exon 4, exon 5, exon 6, exon 7, exon 8, exon 9, intron 1, intron 2, intron 3, intron 4, intron 5, intron 6, intron 7, intron 8, 5' untranslated region, or 3' untranslated region; or

(c) a human BCA3 polynucleotide or polypeptide, where the domain is SH2 site YYSS, SH2 site YSSV, SH2 site YHRG, SH2 site YIEV, SH2 site YPGT, SH2 site YSVT, tyrosine phosphorylation site, RTMAEFMDY, glycosylation site, phosphorylation site, tyrosine phosphorylation motif, SH2-binding motif, open-reading frame, open-reading frame lacking exon 3, open reading frame lacking exon 3 and exon 5, exon 1, exon 2, exon 3, exon 4, exon 5, exon 6, exon 7, intron 1, intron 2, intron 3, intron 4, intron 5, intron 6, 5' untranslated region, or 3' untranslated region.

The polynucleotide encoding the human BCA3 polypeptide is an RNA.

Preferred Polypeptide: The human BCA3, BCA1 and BCA2 comprise a sequence having 210, 154 or 304 amino acids, respectively. These sequences are fully defined in the specification. The fragment of the human BCA1, BCA2 or BCA3 polypeptide comprises at least 5 consecutive amino acids of the human BCA1, BCA2 and BCA3 polypeptide, respectively. The fragment is a portion of the domains cited above. The polypeptide has an amino acid sequence that has at least 90% identity to the fragment described above, or to the amino acid sequences cited above.

Preferred Antibody: The antibody immunospecifically binds to a human BCA polypeptide when bound to a binding partner.

Preferred Methods:

M1 comprises:

(a) contacting a BCA antibody with a sample suspected of containing a BCA polypeptide from the subject, under conditions that allow the BCA antibody to bind the BCA polypeptide; and

(b) detecting or measuring binding of the BCA antibody to the BCA1, BCA2, BCA3, BCA4, BCA5, BCA6 or BCA7 polypeptide.

The BCA-related disorder is determined to be present when the presence or amount of the BCA polypeptide indicated by the detection or measurement of binding differs from a control value representing the amount of BCA polypeptide present in an analogous sample from a subject not having the BCA-related disorder. The stage of a BCA-related disorder in a subject is determined when the presence or amount of BCA polypeptide indicated by the detection or measurement of binding is compared with the amount of BCA polypeptide present in an analogous sample from a subject having a particular stage of a BCA-related disorder, e.g. breast cancer.

M2 comprises:

(a) contacting the BCA polypeptide with an analyte to allow the analyte to bind the BCA polypeptide; and

(b) detecting binding of the BCA polypeptide to the analyte.

M3 comprises:

(a) contacting the BCA polypeptide with a positionally addressable array comprising several proteins, with each protein at a different position on a solid support; and

(b) detecting binding of the BCA polypeptide to a protein on the array.

M4 comprises:

(a) contacting the complex with the analyte to allow the analyte to bind to the complex; and

(b) detecting binding of the BCA polynucleotide or polypeptide to the analyte, where the analyte binds to the BCA polynucleotide or polypeptide when bound to the binding partner, and does not bind to the BCA polynucleotide or polypeptide when not bound to the binding partner.

M5 identifying an analyte that inhibits formation of a complex comprising the BCA polynucleotide or polypeptide comprises:

(a) contacting the complex with the analyte; and

(b) measuring the amount of the complex, where a reduction in the amount of complex indicates that the analyte inhibits formation of the complex.

M6 comprises:

(a) contacting the cell with: (a.1) the BCA polynucleotide; (a.2) the BCA polypeptide; or (a.3) the antibody that immunospecifically binds to the BCA polypeptide; and

(b) measuring cell growth, where an inhibition of cell growth indicates the presence of an inhibitor of growth of a breast cancer cell.

Preparation: The BRA-3 polypeptide is prepared by standard recombinant techniques comprising culturing a cell expressing BRA-3 polynucleotide and isolating the polypeptide (claimed). The polynucleotide, expression vector, cell and transgenic animal are prepared by standard recombinant techniques.

ABEX

UPTX: 20030214

SPECIFIC SEQUENCES - Specifically claimed is a human BCA polypeptide comprising sequences of, for example 210 (BCA3), 154 (BCA1) and 304 (BCA2) amino acids, fully defined in the specification. Also claimed are nucleic acid sequences for example, the nucleotide sequence of the human BCA3 gene, which comprises 1319 bp fully defined in the specification.

ADMINISTRATION - Dosage is 0.01-10 mg/kg/day. Preferably, dosage is 0.01-5 or 10-50 mg/kg, depending on the route of administration. Administration is oral, intravenous, subcutaneous, transdermal, rectal, intramuscular, topical, depo injection, implantation, time-release mode, intracavitary, intranasal, intratumoral, intraocular, or parenteral.

EXAMPLE - More than 1000 cDNA clones for genes that may be activated or inactivated during progression of breast cancer were isolated by subtractive hybridization and differential display methods using matched breast tumor and normal breast cell line RNAs. All cDNA sequences from the breast cancer subtractive cloning library were compared by BLAST to the entire-redundant GenBank database. Seven genes, namely BCA1-7, were identified. Two genes, BCA1 and BCA3 were expressed more highly in abreast tumor tissue than in normal breast tissue.

L46 ANSWER 6 OF 7 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN

AN 2001-071267 [08] WPIX

DNC C2001-019969

TI Novel isolated **Smurf** protein useful for inhibiting bone morphogenic protein or tumor growth factor-beta activation pathway, for treating cancer and to block osteogenesis, hair growth, tooth formation.

DC B04 D16

IN THOMSEN, G H; WRANA, J

PA (HSCR-N) HSC RES & DEV LP; (UYN) UNIV NEW YORK STATE RES FOUND

CYC 93

PI WO 2000077168 A2 20001221 (200108)\* EN 106 C12N000-00

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW MZ  
NL OA PT SD SE SL SZ TZ UG ZW

W: AE AG AL AM AT AU AZ BA BB BG BR BY CA CH CN CR CU CZ DE DK DM EE  
ES FI GB GD GE HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT  
LU LV MA MD MG MK MN MW MX MZ NO NZ PL PT RO RU SD SE SG SI SK SL  
TJ TM TR TT TZ UA UG US UZ VN YU ZA ZW

AU 2000056107 A 20010102 (200121)

EP 1192174 A2 20020403 (200230) EN C07H021-04

R: AL AT BE CH CY DE DK ES FI FR GB GR IE IT LI LT LU LV MC MK NL PT  
RO SE SI

JP 2003502064 W 20030121 (200308) 131 C12N015-09

CN 1409722 A 20030409 (200345) C07H021-04

ADT WO 2000077168 A2 WO 2000-US16250 20000612; AU 2000056107 A AU 2000-56107  
20000612; EP 1192174 A2 EP 2000-941398 20000612, WO 2000-US16250 20000612;  
JP 2003502064 W WO 2000-US16250 20000612, JP 2001-504003 20000612; CN  
1409722 A CN 2000-811354 20000612

FDT AU 2000056107 A Based on WO 2000077168; EP 1192174 A2 Based on WO  
2000077168; JP 2003502064 W Based on WO 2000077168

PRAI US 1999-138969P 19990611

IC ICM C07H021-04; C12N000-00; C12N015-09  
 ICS A01K067-027; A61K031-7088; A61K038-00; A61K039-395; A61K045-00;  
 A61K048-00; A61P019-08; A61P025-00; A61P027-02; A61P043-00;  
 C07K014-00; C07K016-40; C12N001-15; C12N001-19; C12N001-21;  
 C12N005-10; C12N009-00; C12N015-00; C12Q001-02; G01N033-15;  
 G01N033-50; G01N033-53

AB WO 200077168 A UPAB: 20011129  
 NOVELTY - An isolated **Smurf1** or **Smurf2** protein (I), is new.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

- (1) an isolated nucleic acid (II) encoding (I);
- (2) a vector (III) comprising (II);
- (3) a host cell (IV) comprising (III);
- (4) production of (I);
- (5) a transgenic non-human animal that expresses a human (I);
- (6) screening (M) for a modulator of **Smurf** activity, comprising detecting modulation of **Smurf** activity in the presence of a test compound relative to **Smurf** activity in the absence of the test compound;
- (7) an antibody (V) that specifically binds to (I);
- (8) an oligonucleotide or nucleic acid (VI) that specifically hybridizes to (II) under highly stringent conditions; and
- (9) promoting a bone morphogenic protein or transforming growth factor (TGF)- beta activation pathway in a cell, comprising suppressing expression of endogenous **Smurf** in the cell.

ACTIVITY - Cytostatic.

MECHANISM OF ACTION - Negative regulator of Smad signal transduction; antagonist of BMP and TGF- beta signaling pathway.

The inhibition of Smad1 by **Smurf1** was tested. By over expressing Smad1 and Smad2 together with various dosages of **Smurf1** in *Xenopus* animal caps, the ability of **Smurf1** to directly antagonize the mesoderm induction activities of Smad1 and Smad2, was tested. The results showed that expression of Smad1 alone induced ventral mesoderm, as demonstrated by expression of the ventral/posterior mesodermal markers *Xhox3* and *Xcad1*. However, co-expression of **Smurf1** and Smad1 blocked induction of these markers at all **Smurf1** doses tested, demonstrating that **Smurf1** can antagonize Smad1 activity.

USE - Expression of (I) from (III) in a cell is useful for inhibiting a bone morphogenic protein (BMP) or transforming growth factor- beta (TGF beta ) activation pathway in a cell (claimed). (I) is useful to block chondrogenesis, osteogenesis, blood differentiation, cartilage formation, neural tube patterning, retinal development, heart induction and morphogenesis, hair growth, tooth formation, gamete formation and a wide variety of tissue and organ formation processes, and hinder the regeneration, growth, maintenance, etc., of bone and other tissues that are dependent on the BMP pathway. (I) is useful for screening for various drugs and/or antibodies that can either enhance the BMP pathway, or inhibit it by antagonizing or mimicking the activity of (I), respectively, and in screening assays for identifying specific ligands of (I). (I) is useful as an immunogen to generate antibodies that are useful to alter the BMP pathway by inhibiting (I) or for diagnostic purposes. (I) is useful for treating a disorder associated with BMP or TGF- beta activation, such as cancer. (I) or inhibitor of (I) can be delivered by a vector to modulate Smads, e.g. to prevent **Smurf** regulation of Smads where BMP or TGF beta activity is desired, such as in bone regeneration or to study **Smurf** regulator processes in vivo.

Dwg.0/18

FS CPI

FA AB; DCN

MC CPI: B04-C01G; B04-E02F; B04-E03F; B04-E05; B04-E06; B04-E08; B04-F0100E; B04-G01; B04-N02A0E; B04-P0100E; B11-C08; B12-K04E; B14-H01;

B14-H01B; D05-C12; D05-H09; D05-H12A; D05-H12B2; D05-H12D1;  
D05-H12D2; D05-H12E; D05-H14; D05-H14B; D05-H16A; D05-H17A6;  
D05-H17B6

TECH

UPTX: 20010207

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preparation: (I) is produced by growing (IV) under conditions that permit expression of (I) from (III) (claimed). Preferred Protein: (I) is human **Smurf1** or **Smurf2** protein and has a mutation corresponding to C710A or C716A, respectively. Preferred Method: In (M) the **Smurf** activity is ubiquitination of a Smad protein in a host cell or interaction of a **Smurf** WW domain with a PPXY domain of a Smad protein. The test compound is screened for the ability to inhibit the interaction.

ABEX

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WIDER DISCLOSURE - Disclosed as new are the following:

- (1) an antisense nucleic acid which may be used to inhibit expression of **Smurf1** or **Smurf2**, particularly to enhance bone morphogenic protein or TGF-beta signaling pathway;
- (2) analogs, derivatives of (I), homologs from other species, and mutant variants, which have the same or a homologous functional activity, and their production and use; and
- (3) cloning vectors containing genes encoding analogs and derivatives of (I).

SPECIFIC SEQUENCES - (I) comprises at least 10 contiguous residues of a 723 or 748 residue amino acid sequence corresponding to **Smurf1** and **Smurf2**, respectively, and is encoded by (II) with a 2172 or 2247 base pair sequence, all fully defined in the specification (claimed).

EXAMPLE - A *Xenopus* Smad1 cDNA was cloned into the pGBT9 vector and used to screen a *Xenopus* oocyte cDNA library using *Xenopus* Smad1 as the bait protein. A partial cDNA was isolated and used to screen a *Xenopus* Stage9 cDNA library to obtain a full length **Smurf1** cDNA with a 2172 base pair sequence, fully defined in the specification. A human **Smurf1** cDNA encoding all but the first 8 amino acids was identified and used to construct human **Smurf1**. Human **Smurf2** was identified and cloned using a *Xenopus* **Smurf1** sequence. Two overlapping expressed sequence tags (EST) clones corresponding to **hSmurf2** were obtained and used to construct a full length sequence for **hSmurf2**. **Smurf2** was closely related to **Smurf1**, and displayed 75 % homology to the amino acid sequence of **hSmurf1**. Several overlapping human clones displaying similarity to **Smurf1** were identified and a full-length version of **Smurf2** was constructed. A partial mouse **Smurf2** cDNA clone encoding 225 amino acids of open reading frame including the stop codon and displaying 96 % amino acid identity to human **Smurf2** was identified. For mammalian expression constructs of **Smurf2**, the open reading frame was amplified and was subcloned into pCMV5 in frame with an amino-terminal Flag or Myc tag.

L46 ANSWER 7 OF 7 WPIX COPYRIGHT 2004 THOMSON DERWENT on STN

AN 2000-317970 [27] WPIX

DNC C2000-096321

TI Targeting degradation of polypeptide useful for treating cancer and other proliferative disorders, involves conjugating polypeptide with ubiquitin protein ligase or inhibiting ubiquitination using organic compound.

DC B04 D16

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PA (HARD) HARVARD COLLEGE

CYC 87

PI WO 2000022110 A2 20000420 (200027)\* EN 185 C12N015-00

RW: AT BE CH CY DE DK EA ES FI FR GB GH GM GR IE IT KE LS LU MC MW NL

OA PT SD SE SL SZ TZ UG ZW

W: AE AL AM AT AU AZ BA BB BG BR BY CA CH CN CU CZ DE DK EE ES FI GB

GD GE GH GM HR HU ID IL IN IS JP KE KG KP KR KZ LC LK LR LS LT LU  
 LV MA MD MG MK MN MW MX NO NZ PL PT RO RU SD SE SG SI SK SL TJ TM  
 TR TT TZ UA UG UZ VN YU ZA ZW

AU 2000012030 A 20000501 (200036) C12N015-00  
 ADT WO 2000022110 A2 WO 1999-US23705 19991008; AU 2000012030 A AU 2000-12030  
 19991008

FDT AU 2000012030 A Based on WO 2000022110

PRAI US 1998-103787P 19981009

IC ICM C12N015-00

ICS C12N005-10; C12N015-12; C12N015-37; C12N015-52; C12N015-62

ICA C07K014-00

AB WO 200022110 A UPAB: 20000606

NOVELTY - Targeting degradation of a target polypeptide (I) in vivo, comprising ubiquitinating (I) by expressing in a cell, a ubiquitin protein ligase polypeptide (UL), having ubiquitin conjugation activity linked to interaction domain of (I), and recruiting (I) to (UL), is new. The ubiquitin-(I) conjugate formed is targeted for degradation.

DETAILED DESCRIPTION - INDEPENDENT CLAIMS are also included for the following:

(1) a method for decreasing the level of (I), comprising providing an SCF (SKP1, Cullin and F-box containing proteins) recruitment domain operably linked to a (I) interacting domain, to form a fusion protein, and expressing the fusion protein, so that the level of (I) is decreased;

(2) a method for creating a destabilized polypeptide subject to SCF-mediated proteolysis, comprising providing an SCF recruitment domain, and operably linking it to the polypeptide;

(3) a method for expressing a destabilized (I) subject to SCF-mediated proteolysis, comprising providing an SCF recruitment domain operably linked to (I), and expressing the fusion polypeptide;

(4) a nucleic acid (III) for expressing an SCF recruitment domain-interaction domain of (I), comprising a nucleic acid encoding an SCF recruitment domain, and a heterologous polypeptide domain;

(5) a vector (IV) comprising (III);

(6) a cell comprising (IV); and

(7) a method of treating a cell to stabilize a target of ubiquitin protein ligase, comprising contacting the cell with a preparation comprising an organic compound, which can competitively inhibit interaction of the target polypeptide with the ligase.

ACTIVITY - Cytostatic; nootropic; anticonvulsant; antimicrobial. Targeted degradation of endogenous p107 in mammalian cells was tested using beta TrCP-E7N. p107 is a protein related to the retinoblastoma tumor suppressor protein pRB. Cervical carcinoma C33A cells lacking wild type pRB were transmitted with engineered beta TrCP-E7N. The cells were co-transfected with cytomegalovirus (CMV)-CD19 and selected by immunomagnetic selection of cells expressing CD19. The results showed that levels of p107 was significantly decreased in beta TrCP-E7N expressing cells but were not affected in cells expressing the control protein unable to bind p107.

MECHANISM OF ACTION - Ubiquitin-conjugation-regulator; gene therapy.

USE - The methods are useful for decreasing or increasing the level of (I), and for creating and expressing a destabilized (I) which is subjected to SCF mediated proteolysis (claimed). Degrading any desired protein in a cell is useful for preventing or treating diseases caused by the presence of abnormal amount of the specific polypeptides, for drug discovery and for gene therapy. Diseases treated include cancer, by degradation of oncoproteins, Huntington's disease, other proliferative disorders and microbial infections.

ADVANTAGE - The method provides a quick, easy and economic alternative to gene knockout technology. (I) can be degraded at all stages, or a specific stage, of development in the mature animal.

Dwg.0/0

FS CPI

FA AB; DCN

MC CPI: B04-A08C2E; B04-E02F; B04-E08; B04-F0100E; B04-P01A0E; B11-C08E1;  
B11-C09; B14-A01; B14-A02; B14-A03; B14-A04; B14-H01B; B14-J01A4;  
B14-J07; B14-S03; D05-H09; D05-H12C; D05-H12E; D05-H14; D05-H16;  
D05-H17C

TECH UPTX: 20000606

TECHNOLOGY FOCUS - BIOTECHNOLOGY - Preferred Polypeptide: The ubiquitin-target polypeptide conjugate further undergoes ubiquitin-dependent proteolysis by proteasomes. (UL) is an E3 ubiquitin protein ligase, preferably an SCF, HECT or a UBR1 polypeptide. The SCF polypeptide is selected from Skp1 or a cullin polypeptide or an F-box polypeptide, preferably Cdc4p, Pop1p, Pop 2p, Grr1p, Met30p, HOSp, beta TrCpP or FWD1p which further comprises a WD domain. The HECT polypeptide is E6-Ap, Nedd-4, RSP5, Smurf1, TOM1 or EDD. E3 ubiquitin protein ligase is a yeast or a mammalian UBR1 polypeptide. (I) is a retinoblastoma, a p107, IkappaB, Sic1p, Cln2p, E2 or beta catenin polypeptide. The interaction domain of (I) is either a papillomavirus E7 or a SV40 LTP polypeptide. (II) is a peptide or a peptidomimetic which is a competitive inhibitor of a WD domain comprising a general chemical formula: G-H-X(3-6)-h-X-X-h-X-r-X-t(2-3)-p-X-h-h-X-X-X-X-D-X-X-X-X-h-W-D.

ABEX UPTX: 20000606

WIDER DISCLOSURE - The following are disclosed as new:

- (1) transgenic plants and animals comprising (III);
- (2) a kit comprising (III); and
- (3) methods and compositions for the identification of inhibitors of the interaction between an F-box protein and other subunits of an SCF complex.

SPECIFIC SEQUENCES - The F-box polypeptide has a 721, 541, 1141, 601, 661 or 541 residue amino acid sequence, encoded by a 20341(Cdc4p), 2101(hbetaTrCp), 4441(Grr1p), 33901(Met30p), 2101(Pop2) or 2161(FWD1p) base pair sequence (claimed). All sequences fully defined in the specification.

ADMINISTRATION - The administration is oral, buccal, parenteral, rectal, systemic, topical or local routes. No specific dosage is given.

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(FILE 'HOME' ENTERED AT 09:44:07 ON 21 SEP 2004)  
SET COST OFF

FILE 'REGISTRY' ENTERED AT 09:44:26 ON 21 SEP 2004  
E SMURF

L1 20 S E3-E5 OR ?SMURF?/CNS

E SMAD

L2 403 S E3-E21

FILE 'HCAPLUS' ENTERED AT 09:46:07 ON 21 SEP 2004

L3 15 S L1

L4 85 S ?SMURF?

L5 90 S L3,L4

L6 9 S L5 AND L2

L7 48 S L5 AND ?SMAD?

L8 50 S L6,L7

L9 42 S L8 AND UBIQUITIN?

L10 50 S L8,L9

L11 7 S L10 AND SCREEN?

E DRUG SCREENING/CT

L12 24987 S E3-E5

L13 6373 S E9,E10

E E3+ALL

L14 31124 S E9,E8

E E12+ALL

L15 9001 S E10  
L16 3897 S E21+NT  
L17 5 S L10 AND L12-L16  
L18 7 S L11,L17  
L19 8 S L10 AND ?MODULAT?  
L20 11 S L5 AND ?MODULAT?  
L21 15 S L18-L20  
L22 0 S L10 AND ?PPYX?  
L23 7 S L10 AND WW (L) DOMAIN  
L24 21 S L21,L23  
E WRANA J/AU  
L25 117 S E3-E9  
E THOMSEN G/AU  
L26 35 S E3-E6  
L27 9 S L25,L26 AND L5  
L28 25 S L24,L27  
L29 18 S L5 AND (PD<=19990611 OR PRD<=19990611 OR AD<=19990611)  
L30 2 S L28 AND L29  
L31 0 S L5 AND (PY<=1999 OR PRY<=1999 OR AY<=1999) NOT L29  
L32 9 S L27,L30  
L33 16 S L29 NOT L32  
SEL DN AN 2-9 11-16  
L34 2 S L33 NOT E1-E38  
L35 11 S L32,L34  
L36 3 S L3 AND L29  
L37 11 S L35,L36

FILE 'HCAPLUS' ENTERED AT 09:57:27 ON 21 SEP 2004

FILE 'BIOSIS' ENTERED AT 09:57:45 ON 21 SEP 2004

E WRANA J/AU  
L38 142 S E3-E8  
E THOMSEN G/AU  
L39 45 S E3,E5,E9,E10  
L40 60 S ?SMURF?  
L41 6 S L38,L39 AND L40  
L42 50 S L38,L39 AND ?SMAD?  
L43 5 S L41 AND L42  
L44 1 S L41,L43 AND PY<=1999

FILE 'BIOSIS' ENTERED AT 10:00:17 ON 21 SEP 2004

FILE 'WPIX' ENTERED AT 10:00:51 ON 21 SEP 2004

L45 8 S L4/BIX  
L46 7 S L45 NOT THREAD/TI

FILE 'WPIX' ENTERED AT 10:01:58 ON 21 SEP 2004

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